بسم الله الرحمن الرحيم

Sleep Deprivation, Cardiovascular Effects and the Role of Anesthesiologists

Sleep and anesthesia have some common or "overlapping" neural pathways. Both involve wakefulness; while they are not the same; anesthesia is an iatrogenic, reversible, pharmacologic-based coma; which could affect the CNS neural pathways at many levels. In the current era of modern anesthesiology, the practice and science of anesthesia is composed of 4 basic elements; (1):

1. hypnosis (i.e. iatrogenic pharmacologic-induced coma)
2. amnesia (not to remember the events of the operation)
3. analgesia (being painless)
4. akinesia (lack of movements to stimuli)

The first two ingredients of anesthesia could have common points with sleep. Thalamic nuclei are involved both in sleep and anesthesia (2, 3); though, they are not the same phenomena (4). However, could there be any clinical concern if some of our patients have abnormalities in sleep?

In fact, the effects of sleep deprivation have long been studied in patients undergoing anesthesia for surgical operations (4, 5). Sleep deprivation causes altered neurohumoral activity, neuroendocrine dysregulations, abnormalities in the immune system and impairments in cardiac autonomic function (6, 7). Sleep deprivation may affect the clinical effects of the anesthetics or it may create unpredicted changes in the clinical response to a determined dose of anesthetic drugs (8).

In this volume of the Journal, Choopani et al have published their results regarding sleep deprivation; they have demonstrated that in rats, if sleep deprivation is induced prior to an ischemia/reperfusion event, it can increase the chance for ventricular tachycardia and ventricular fibrillation; also, they have shown that this untoward effect could be eliminated using chemical sympathectomy (9).

In clinical practice, the main message from this study could be that when anesthesiologists perform anesthesia for their patients, they should be aware of effects of acute or chronic sleep deprivation. Undoubtedly, sleep deprivation could occur during the perioperative period or maybe a longer accompanying abnormality in patients undergoing anesthesia for surgery. However, the unwanted cardiovascular effects are really of great importance. As Choopani et al have quoted the following are among the other unwanted effects of sleep deprivation which mandate our vigilance in these patients (9):

- hypertension
- over activity of the sympathetic nervous system
- increased heart rate
- vasoconstriction
- salt retention

Also, based on this study and other related studies (10-12), chemical sympathectomy could be an efficient method to relieve these effects. Preparedness against this phenomenon remains an important concern.

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Original Article

Effects of Mesenchymal Stem Cells Conditioned Medium on Behavioral Aspects of Inflammatory Arthritic Pain Induced by Complete Freund's Adjuvant

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Abstract

Background: Rheumatoid arthritis is a type of inflammatory pain and is an autoimmune and chronic inflammatory disease which can lead to hyperalgesia, edema and decreased motor activity in affected area. Mesenchymal stem cells conditioned medium (MSC-CM) has anti-inflammatory mediators which can regulate the immune responses, alleviate inflammatory symptoms and has a paracrine effects too. The aim of this study was to evaluate the effects of mesenchymal stem cells conditioned medium on behavioral aspects of inflammatory arthritic pain which induced by Complete Freund's adjuvant (CFA).

Materials and Methods: Complete Freund's adjuvant-induced arthritis (AA) was caused by single subcutaneous injection of CFA into the rat's hind paw on day zero. MSC-CM was administered daily and intraperitoneal during the 21 days of the study after CFA injection. Hyperalgesia and edema were assessed on days 0, 7, 14 and 21 of the study respectively with radian heat and plethysmometer instrument.

Results: The results of this study indicated the significant roles of MSC-CM in betterment of inflammatory symptoms such as hyperalgesia and edema during different stages of inflammation caused by CFA. The continuing injection of MSC-CM could reduce the inflammatory symptoms.

Conclusion: Long term treatment by MSC-CM can alleviate hyperalgesia and edema and decrease those to the level of the time before induction of inflammation.

Keywords: Inflammation, Pain, Hyperalgesia, Edema, MSC-CM

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Introduction

Inflammatory pain is a type of pathological pain caused by peripheral tissue inflammation and tissue damage which is characterized by an increased sensitivity to stimuli of the affected tissue. Rheumatoid arthritis (RA) is a type of inflammatory pain (1) and is an autoimmune inflammatory disease with unknown etiology which has been lead to pain, swelling, hyperalgesia, dryness and inflammation of the joints and surrounding tissue, cartilage and bone destruction, dysfunction and disability (2). The RA

disease process is variable, often is associated with periods of relapse and sometimes with a reduction in symptoms. Despite the unknown etiology of RA, it seems that pro-inflammatory cytokines elevated during the disease which can cause inflammatory symptoms such as hyperalgesia and edema (3). Inflammatory biochemical factors such as the kinins, prostaglandins, histamine, and serotonin act synergistically to induce hyperalgesia as well as increased vascular permeability and also development of acute pain and edema (4). Hyperalgesia caused due to excessive sensitivity of pain receptors because of decrease of activation threshold as a result of release of some chemicals like histamine, bradykinin and etc. The most important cause of progression of hyperalgesia is activation of pain afferents during inflammation. It seems that the neural mechanism of hyperalgesia is sensitization of the primary afferent nociceptive fibers (5). Following inflammation and injury, an inflammatory response is generated by T-lymphocytes and this is further amplified by producing pro-inflammatory cytokines like TNF-α and IL-1β and maintain the development of pain, hyperalgesia and edema (1). A variety of factors like Complete Freund's Adjuvant (CFA), Formalin, Carrageenan and Capsaicin are used to create an inflammatory pain model and there are differences in underlying mechanisms and clinical symptoms of inflammation induced by these adjuvants. Studies have shown that inflammation induced by CFA is usually more stable than other inflammatory adjuvants. Inflammatory pain model induced by CFA is a biphasic model that in the first phase (inflammatory phase) is associated with increased pain due to the presence of inflammatory cytokines such as TNF-α and IL-1β, while in the second phase (arthritic phase) hyperalgesia due to the elevation of opioid receptors expression dramatically decreased (6). Mesenchymal stem cell (MSC) is a population of pluripotent adult stem cells which have a high capacity for self-renewal and proliferation and represent functional characteristics which have open the way for cell-based therapy for autoimmune disorders (7-9). MSCs are able to secrete a wide range of trophic factors which can demonstrate paracrine effects on other cell types. Hence, mesenchymal stem cell conditioned medium (MSC-CM) have shown potential therapeutic applications in regeneration or in pain alleviation (8). MSC-CM is a supernatant that is achieved from *in vitro* cultured stem cells conditions and can cause effects similar to MSC. In recent years their eventuality to take part in the immune response modulation is discussed (9). The exact mechanisms of immune regulation of MSC-CM are not clear yet, but interactions with other immune regulatory cells, elevation of anti-inflammatory mediators and diminution of pro-inflammatory cytokines may be involved in this process (8, 9). This has led to the expansion of novel applications of MSC-CM for the remedy of inflammatory and degenerative rheumatic diseases such as RA, osteoarthritis (OA) and also bone and cartilage disorders (10). Then, based on the potency of MSC-CM in modulation of immune responses and inflammatory symptoms, in this study, the effects of mesenchymal stem cells conditioned medium on behavioral aspects of inflammatory arthritic pain induced by CFA adjuvant was investigated.

Methods

Laboratory animals

In this study, the adult male Wistar rats (n=96) weighing 200–220 gr. were used in all experiments. These animals were housed in polypropylene cages under standard environmental circumstances (22±2ºC, humidity 60–70 %, 12 h light/dark cycle) and allowed free access to food and water. All the experiments were approved by the international association for the study of pain and local ethics committee of the utilization of animals in research for the treatment of animals and the guidelines of the ethical standards for the investigations of experimental pain in animals were followed precisely (11).

In order to determine the effect of MSC-CM on inflammatory pain model and the effectiveness of this treatment, a series of experiments were performed. Rats were randomly divided into different experimental groups, as follows: (a) CFA group, (b) CFA control group, (c) CFA+CM (d) Sham group. According to the study procedure, each group was divided into four subgroups based on different time points of the study (days 0, 7, 14, and 21) and there were 6 rats in each subgroup.

Experimental procedure

Complete Freund's adjuvant (CFA)-induced arthritis was evoked by single subcutaneous injection of (100µL) heat-killed Mycobacterium tuberculosis suspended in sterile mineral oil (10 mg/mL; CFA; Sigma, St Louis, MO, USA) into the rats' right hind paw on day zero (under light anesthesia with methoxyflurane). The CFA control group was received sterile mineral oil once only (100µL) (S.C.). First day after CFA injection, unilateral inflammation was established in the injected hind paw of rat (acute phase), and the next weeks after inflammatory phase were arthritic phase (chronic phase). From the first day after CFA injection, experimental groups received the MSC-CM on a daily basis and were injected intraperitoneal (250µL/rat) (i.p.). The sham group received sterile mineral oil with single subcutaneous (100µL) injection in the rat's right hind paw and also received MSC-CM on a daily basis (250µL/rat) (i.p.). In this study, hyperalgesia and paw edema were assessed on day 0 (immediately before CFA injection), on days 7 (inflammatory phase), 14 and 21 (arthritic phase) (12).

MSC-CM preparation and administration

Bone marrows were obtained from the bone marrow of femurs and tibias of two-month-old male Wistar rats (weighing 200-250 g) under sterile circumstances. The derived tibia and femur bone marrow soaked in cold PBS and eliminated adherent soft tissues. Bone marrow (includes hematopoietic stem cells and stromal cells) were cultured in minimal essential medium alpha (α-MEM, Gibco, Invitrogen, Carlsbad, CA, USA) containing 15% fetal bovine serum (FBS, Gibco, Invitrogen, Carlsbad, CA, USA) and 1% Penicillin/streptomycin (Gibco, Invitrogen, Carlsbad, CA, USA) and incubated at 37°C in the presence of 5% carbon dioxide. The cells reach to passages three were applied for subsequent studies. After 48 hours, the medium of cells were replaced. The medium used in this step is free of FBS. In the next stage, after 48 hours, the medium in flask (MSCs supernatant) were gathered and thereupon filtered by the 0.2 micrometers. Cell supernatant was reserved at -80°C until the injection (13).

Assessment of CFA-induced arthritis and paw edema

To confirm the correct injection of CFA, paw volume was measured in both injected and contralateral paws pre and post-injection during different time points of the study. This measurement was conducted by displacement of an electrolyte solution in a plethysmometer (model 7141; Ugo Basile, Comerio-Varese, Italy). Briefly, the rat hind paw was submerged up to the tibiotarsal joint into the electrolyte-filled Perspex cell of the plethysmometer. The volume of the liquid displacement, which is associated with the paw volume, was illustrated on a digital display. Volume measurements were performed twice for each paw and the average was calculated. The edema was quantified by measuring the differences in the paw volume between the day 0 and other different time points of the study (14, 15).

Behavioral test (Thermal hyperalgesia assessment)

Paw withdrawal latencies (PWL) from noxious heat using the plantar test were assessed in both CFA-injected and control paw on Days 0 (before injection of CFA), 7, 14, and 21. PWL in response to radiant heat by plantar test apparatus was executed in the control and experimental groups (Ugo Basilar, Verse, Italy). 10-15 minutes before the test, rats were placed in a Plexiglas boxes positioned on a glass surface in order to habituate to the test environment. The heat source (infrared light) was positioned under the plantar surface of the affected hind paw and projected focally. The digital timer connected to the heat source recorded the PWL to the nearest 0.1 s. automatically. Heating was terminated at 20 s cut off to prevent tissue damage if an animal failed to withdrawals paw from the stimulus prior to the cut off. Each rat received three trials per hind paw at an interval of 5–10 min. PWL, for each paw at an interval of 5 - 10 min, was done and the results are represented as the difference in the PWL score between injected paw and control paw and statistical comparisons between groups assessed this difference. The value obtained in the negative expressed the hyperalgesia in the inflamed paw (6, 11).

$$H = \frac{(Rt1 + Rt2 + Rt3)}{3} - \frac{(Lt1 + Lt2 + Lt3)}{3}$$

H: The difference between the right and the left response time (Hyperalgesia).

R_t: Right PWL L_t: left PWL

Statistical analysis

Results were represented as mean±standard

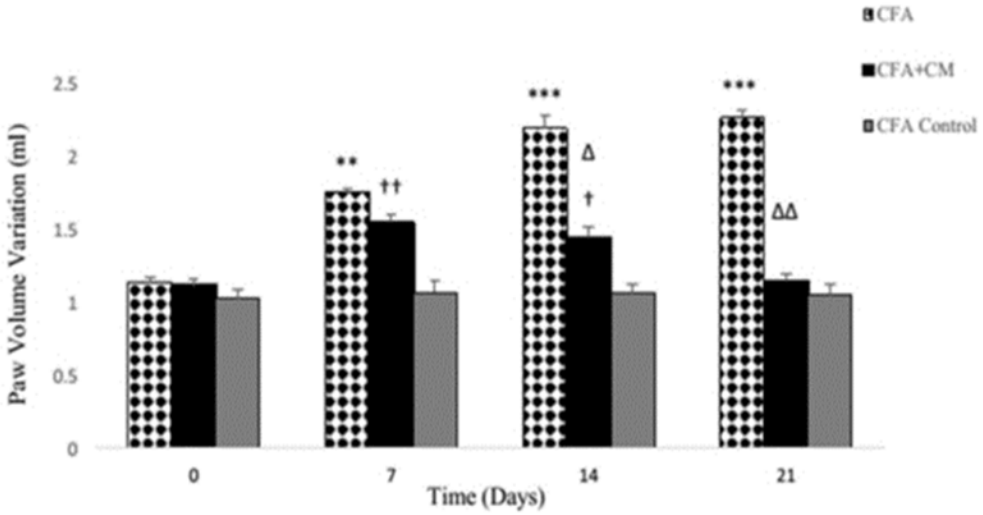

Fig. 1. Paw volume incremented in CFA group in different time points of the study compared to baseline substantially. Long-term injection of MSC-CM downturned paw volume significantly. Results presented as Mean±SEM (n=6/group).
** p≤0.01 and *** p≤0.001 for comparing the paw edema variations between baseline and different days of the study in CFA group. † p≤0.05 and †† p≤0.01 for comparing the paw volume variations between day 0 and different days of the study in CFA+CM group. Δ p≤0.05 and ΔΔ p≤0.01 for illustrating the variations in paw volume at day 14 and day 21 compared to day7 in the CFA+CM group.

error of mean (SEM). To compare the results within the groups (during different time points), repeated measurement ANOVA test (One way ANOVA) and post hoc Tukey multiple range tests were used and unpaired student t- test was applied to identify significant differences of the means on the same days between groups. SPSS software version 21 was used for data analysis and the charts were analyzed by Excel. Statistical significance was accepted at p≤0.05 level.

Results

Paw volume variations during different stages of inflammation

CFA injection in the rats hind paw can induce inflammation and increased ipsilateral paw volume (in the affected paw), which continued up to 21 days after CFA injection. Arthritis due to CFA injection was evaluated by measurements of paw volumes pre- and post-injection (on days 0, 7, 14 and 21). Paw volume significantly increased on days 7, 14, and 21 after CFA injection compared with day 0 and with CFA control group (p≤0.01 for day 7 and p≤0.001 for day 14 and 21). Paw volume on days 7, 14 and 21 compared with day 0 in CFA group showed a considerable increase (p≤0.01 for day 7 and p≤0.001 for day 14 and 21). There were no significant differences in reduction in paw volume following injection of sterile mineral oil to the rat's right hind paw on different days of the study relative to baseline in the CFA control group. Additionally, there were no significant differences in paw volume of rats during 21-days of the study in sham group (Hence, the results of the sham groups are not shown graphically).

Daily administration (i.p.) of MSC-CM in the CFA+CM group could reduce paw volume. MSC-CM injection in CFA+CM group could significantly reduce paw edema throughout this study compared to same days in CFA group (p≤0.05 for day 7, p≤0.01 for day 14 and p≤0.001 for day 21). In comparison between the groups CFA+CM and CFA, the results illustrated that reduction of paw edema following the injection of MSC-CM, at day 21 was higher than day 14 substantially (p≤0.01). Continuing injection of MSC-CM decremented paw volume in CFA+CM group, so that at day 21 of the experiment, no

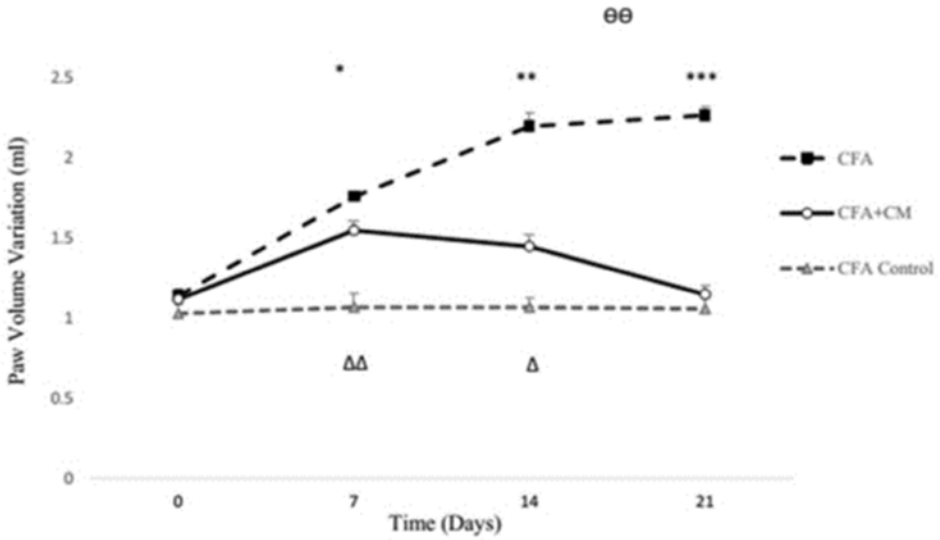

Fig. 2. CFA injection increased paw volume significantly, while MSC-CM administration caused a notable diminution in paw volume compared with CFA group. Results stated as Mean±SEM (n=6/group).
* p≤0.05, ** p≤0.01 and *** p≤0.001 for comparing the paw volume changess between CFA and CFA+CM groups in identical days. Δ p≤0.05 and ΔΔ p≤0.01 for comparing the paw volume variations between CFA+CM and CFA control groups in identical days. ΘΘ p≤0.01 for comparing the differences in paw volume alterations in CFA and CFA+CM groups at day 14 compared to day 21.

significant distinction in paw volume in CFA+CM group compared with CFA control group was perceived.

Variations in thermal hyperalgesia during different stages of inflammation

Intraplantar injection of CFA into the right hind paws of rats caused inflammation and hyperalgesia in the affected paw, which continued up to 21 days after CFA injection. Arthritis due to CFA injection was evaluated by measurements of hyperalgesia pre- and post-injection (on days 0, 7, 14 and 21). Hyperalgesia significantly increased on the 7th day in the CFA group compared with day 0 (p≤0.001), but the persistence of inflammation significantly decreased hyperalgesia on later two days of the study (days 14 and 21) compared to baseline. Howbeit, there was still a significant increase compared to day 0 (p≤0.01). There were no significant differences in reduction of thermal hyperalgesia following injection of sterile mineral oil to the rat's right hind paw on different days of the study relative to baseline in the CFA control group. Considerable difference in the PWL between CFA and CFA control groups on different time points of the study were perceived (p≤0.001). Additionally, there were no significant differences in PWL of rats during 21-days of the study in sham group (Hence, the results of the sham groups are not shown graphically).

Daily administration (i.p.) of MSC-CM in the CFA+CM group declined hyperalgesia, as the continuity of this injection for 21 days also decremented hyperalgesia even more than the baseline (p≤0.05). MSC-CM injection in CFA+CM group significantly declined thermal hyperalgesia throughout this study compared to same days in CFA group (p≤0.05 for day 7 and p≤0.001 for days 14 and 21) and the continued injection for 21 days reduced thermal hyperalgesia even more than CFA control group. In comparison between the CFA and CFA+CM groups, the results illustrated that the rate of reduction of thermal hyperalgesia following the injection of MSC-CM, at day 21th after CFA injection between these two groups was higher than days 7 and 14 substantially (p≤0.001). Continuing injection of MSC-CM decremented PWL in CFA+CM group, so that at day 21 of the experiment, no significant difference in PWL in CFA+CM group compared with

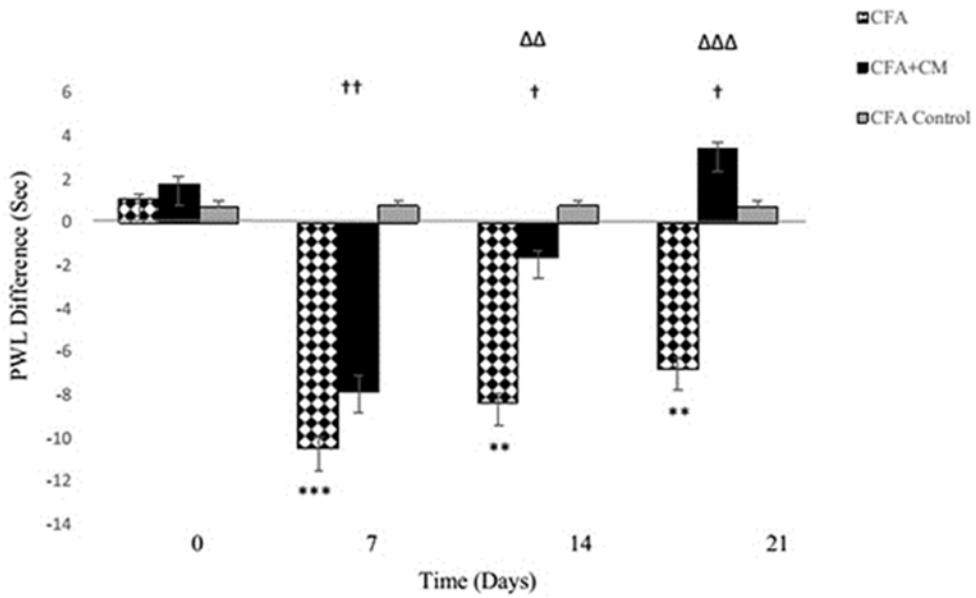

Fig. 3. Thermal hyperalgesia substantially incremented in different time points of the study in CFA group compared to day zero and long-term injection of MSC-CM considerably decremented thermal hyperalgesia. Results presented as Mean ± SEM (n= 6/group).
** p≤0.01 and *** p≤0.001 for comparing the PWL alterations in CFA group between day 0 and different time points of the study. † p≤0.05 and †† p≤0.01 for comparing the PWL variations in CFA+CM group between day 0 and different time points of the study. ΔΔ p≤0.01 and ΔΔΔ p≤0.001for comparing the changes in PWL in the CFA+CM group at day 14 and day 21 compared to day 7.

CFA control group was observed.

Discussion

The main objective of the present study was to evaluate the effects of mesenchymal stem cells conditioned medium on behavioral aspects of inflammatory pain induced by CFA adjuvant. We also assessed hyperalgesia and edema during the long-term administration of MSC-CM.

The results of this study indicated the role of MSC-CM in reducing edema and hyperalgesia during different stages of inflammation caused by CFA adjuvant. The continuing injection of MSC-CM could reduce the inflammatory symptoms to a time before induction of inflammation. Therefore, the CFA+CM group on day 21 of the study did not have significant difference with baseline and the control group.

In this study plantar injection of CFA induced paw inflammation and edema which continued up to day 21 after the CFA injection. Hyperalgesia elevated considerably on day 7 after the CFA injection, but the continuity of inflammation declined hyperalgesia notably on days 14 and 21 of the study compared to day 7. Animal models of inflammatory and neuropathic pain are widely applied to study the mechanisms of acute and chronic pain (16). Arthritis model induced by CFA (containing inactivated Mycobacterium tuberculosis bacteria which is suspended in sterile mineral oil) in rats is an inflammatory model extensively used in etiopathogenic investigational drug and molecular studies due to its similarity to human RA and assay the pathophysiological and pharmacological changes during human RA (11).

Intraplantar injection of inflammatory agents such as CFA causes elevated firing of peripheral afferents in the spinal cord, leading to hyperalgesia (17). Inflammatory pain is specified by an increased sensitivity to mechanical or thermal stimuli of the affected area. Subsequent of tissue injury, an inflammatory response is initiated by local macrophages and reinforced by migrating blood cells (1). Sensitization of the primary afferent nociceptive fiber during inflammation is an important factor in the

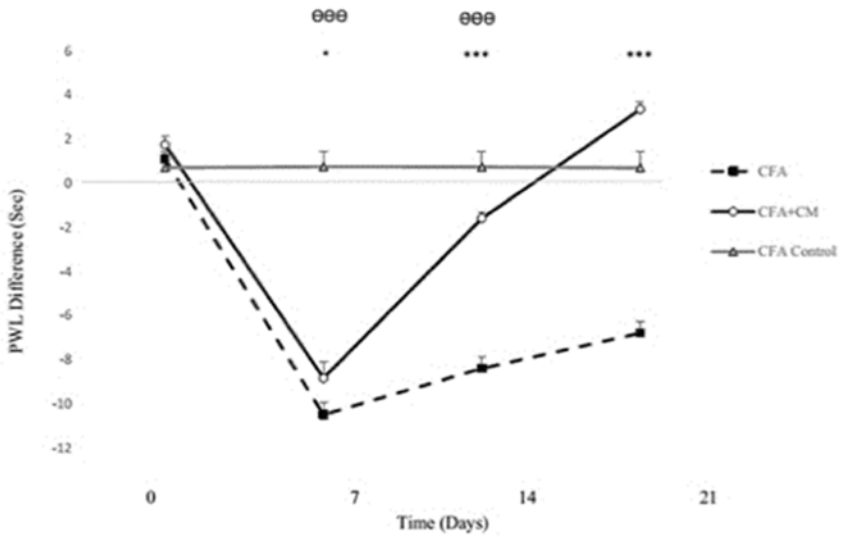

Fig. 4. CFA injection considerably incremented thermal hyperalgesia while MSC-CM administration reduced thermal hyperalgesia compared to the CFA group substantially. Results stated as Mean±SEM (n=6/group). * $p \leq 0.05$ and *** $p \leq 0.001$ for Comparing the PWL variations between CFA and CFA+CM groups in identical days. ΘΘΘ $P \leq 0.001$ for comparing the differences in PWL variations in CFA and CFA+CM groups at days 7 and 14 compared to day 21.

creation and development of hyperalgesia (5, 6).

The behavioral tests as an example hyperalgesia, following inflammatory pain conditions examine the changes in an animal's responses to mechanical or heat stimuli and reflect an alteration of sensory processes. These behavioral tests have been effectual for elucidating the mechanisms of central and peripheral processes which occur in response to inflammation (16).

Pursuant to Hargreaves study, rats due to its inflammation induced by plantar injection of CFA, had withdrawn his affected paw from the thermal stimuli, but some other studies have shown that motor behaviors subsequent of the inflammation are normal and no notable changes in motor activities are observed (18).

A study illustrated that plantar injection of CFA incremented hyperalgesia and edema from 24 hours after the CFA injection and continued up to the first week after the injection (14). However, Cicala *et al*, showed that hyperalgesia existed only between Days 14 and 21 subsequent of arthritis induction due to CFA injection (19). Other studies have indicated that hyperalgesia and edema induced by plantar injection of CFA two hours after the injection are initiated and continued for at least a month. The researchers expressed that injection of inflammatory factors such as CFA to the hind paw through lessen the stimulation threshold of peripheral afferents in the spinal cord lead to hyperalgesia and edema during the first weeks after intervention (6, 11, 20).

Thermal hyperalgesia never happened in the contralateral paw during arthritis induced by CFA adjuvant. Injection of CFA into the one hind paw may have a pivotal role in the induction of hyperalgesia which occurs only in the ipsilateral paw, however the reasons for the absence of hyperalgesia in the contralateral paw should be clarified (6).

The results of this study in line with previous studies have shown that inflammatory model induced by CA injection, is a biphasic model that in the first phase (acute phase) was associated with increase of pain and hyperalgesia due to the presence of pro-inflammatory cytokines such as TNF-α and IL-1β while in the second phase (chronic phase), hyperalgesia substantially declined compared to the previous days due to the presence of opioid receptors (6). Hammond et al., represented that an increase in the potency of opioid agonists alleviates hyperalgesia as an example of inflammatory symptoms through inflammation and arthritis (21). Studies have suggested that MSC-CM containing multiple growth

factors, anti-inflammatory mediators and different chemokines that can alleviate inflammatory symptoms and problems subsequently be involved.

Scientists showed that MSC-CM not only through production of soluble trophic factors can stimulate intracellular mechanisms of damaged cells and inflammatory area but also by inducing secretion of functional active agents via neighboring cells can relieve symptoms of inflammation (8, 22, 23). accordingly, our results not only represent the effectual role of MSC-CM in the betterment of symptoms of acute phase of the inflammation induced by CFA, but also showed that continuing the administration of MSC-CM during inflammation could reduce inflammatory symptoms until day 21 of the study (chronic arthritic phase).

Conclusion

Our study showed that MSC-CM long term treatment significantly reduced hyperalgesia and paw edema during both acute and chronic phases of CFA-induced inflammatory arthritis in male wistar rats. However, further studies are needed to evaluate the effect of MSC-CM on different aspects of inflammation, cytokines production and intracellular signaling pathways activity.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Original Article

Chronic Sleep Deprivation and Ventricular Arrhythmias: Effect of Sympathetic Nervous System

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Abstract

Background: Chronic sleep deprivation through activation of sympathetic nervous system leads to destructive effect on different body organs. We assessed the effect of chronic sleep deprivation on incidence of ischemia/reperfusion-induced ventricular arrhythmias (ventricular tachycardia and ventricular fibrillation) and the role of the sympathetic nervous system in this respect.

Materials and Methods: A total of 24 Rats were randomly divided into four groups of six; 1) ischemia/reperfusion group (IR): 30 minutes ischemia followed by 60 minutes reperfusion was induced, 2) control group (CON): rats has been placed in large multiple platforms for 72h prior to ischemia and reperfusion, 3) Chronic sleep deprivation group (SD): 72h sleep deprivation was induced by using small multiple platform prior to ischemia and reperfusion, 4) Sympathectomy group (SYM): chemical sympathectomy was done 24h before to chronic sleep deprivation and then underwent ischemia and reperfusion. The heart isolated and perfused by langendorff apparatus. After thoracotomy and aorta cannulation, the hearts perfused in the langendorff apparatus using krebs-Henseleit buffer. Hearts were allowed to recovery for 15 min. After recovery period, 15 minutes was considered as baseline prior to 30 minutes ischemia followed by 60 minutes reperfusion. Two thin stainless steel electrodes fixed on the ventricular apex and right atrium for recording the lead II of electrocardiogram (ECG).

Results: There were no significant differences in heart rates between groups, and ventricular tachycardia significantly increased in chronic sleep deprivation group as compared with IR group in ischemia period. Sympathectomy significantly reduced ventricular tachycardia incidence when compared with SD. There is no difference in incidence of ventricular tachycardia between control group and IR group. The incidence of ventricular fibrillation during early reperfusion was significantly augmented ($p<0.05$) in sleep deprivation group as compared with IR group and Sympathectomy significantly could reverse ventricular fibrillation incidence to IR group level as compared with SD group ($p<0.05$).

Conclusion: Induction of 72h sleep deprivation prior to ischemia and reperfusion increased the probability of ventricular tachycardia and ventricular fibrillation occurrence during ischemia and reperfusion and chemical sympathectomy could eliminate this effect.

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Introduction

Sleep as a physiological process affects different biological systems. Its integrity is necessary for maintaining health and homeostasis in human. Autonomic nervous system (ANS), has a critical role in different sleep stages (1). Insufficient sleep is a common problem in modern society, and it has been estimated that about one in five adults are affected by sleep problems (2). Chronic sleep restriction has adverse effects on cardiovascular system, immune responses, hormonal pathways, and thermoregulation. Human studies represent that sleep deprivation can increase activation of the autonomic nervous system (ANS), the hypothalamic-pituitary-adrenal axis (HPA), and the immune system. Sgoifo *et al*, reported that after 48-h sleep deprivation, heart rate and HPA activity considerably augmented. Some studies have shown that 48h sleep deprivation cause imbalance between parasympathetic and sympathetic tones which changes electrocardiographic patterns (3)**.** Sleep deprivation by increasing sympathetic activity leads to increase in percent low-frequency and a decrease in percent high-frequency component of heart rate variability (HRV), increase in low-frequency band of blood pressure variability (BPV), and increase in serum norepinephrine as well as a reduction in maximum endothelial dependent vasodilation. Also five night of partial sleep deprivation is adequate factor to significant increase in sympathetic activity and venous endothelial dysfunction (4).

Despite growing advances against heart disease over the past 50 years, myocardial infarction remains a leading cause of death in united states (5). The development of ventricular arrhythmias and the loss of myocardial contractility are all relevant as clinical consequences of occlusive coronary disease (6). Restitution of the blood supply to an ischemia area that is known as myocardial reperfusion in addition to its cardioprotective effect, can cause myocardial injuries arrhythmias and contractile dysfunction (7).

The autonomic nervous system plays key role in the regulation of cardiac electrophysiology and arrhythmogenesis. The mechanisms by which autonomic activation can induce arrhythmogenic or antiarrhythmic effects are complex and are different for each type of arrhythmias (8). It was found that stimulation of the vagus nerve in rats, dogs, and cats, can prevent ventricular arrhythmias during myocardial ischemia associated with enhanced cardiac sympathetic activity (7).

Since little is known about potential properties of chronic sleep deprivation to facilitate incidence of ventricular arrhythmias, we hypothesized that chronic sleep deprivation through increasing the sympathetic nervous system leads to increase the incidence of ventricular of tachycardia (VT) and ventricular fibrillation during ischemia and reperfusion.

Methods

Experimental animals and ethical approval

Adult Male wistar rats with body weight between 250-300 gr. were housed in an animal room with 12h light/dark cycle at 22±2°C and free access to food and water. All experiments were conducted in accordance with the institutional guidelines of Tehran University of Medical Sciences (Tehran, Iran) and the National Institutes of Health guidelines for the care and use of laboratory animals.

Surgical procedure for heart isolation

Rats were anesthetized with pentobarbital sodium (50 mg/kg; intra-peritoneal) and administered heparin sodium (500 IU; intra-peritoneal). After thoracotomy and aorta cannulation, the hearts perfused in the langendorff apparatus using krebs-Henseleit buffer containing: $NaHCO_3$ 25; KCl 4.7; NaCl 118/5; $mgso_4$ 1/2; KH_2PO_4 1/2; glucose 11; CaCl 2/5. The perfusion pressure was maintained at 70cm H_2O. The perfusate was bubbled with a 95% O_2 and 5% CO_2 gas mixture, and the bubbling rate was adjusted to maintain a physiological PH (7/35-7/45). The perfusate temperature was maintained at 37°c. Tow thin stainless steel electrodes fixed on the ventricular apex and right atrium for recording the

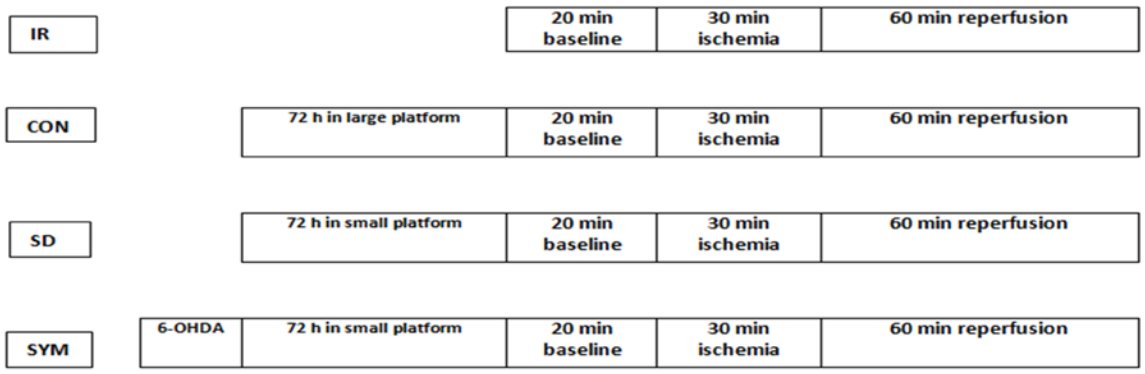

Fig. 1. Schematic diagram of the experimental protocol. IR: ischemia-reperfusion, CON: Control, SD: sleep deprivation, SYM: sympathectomy**.**

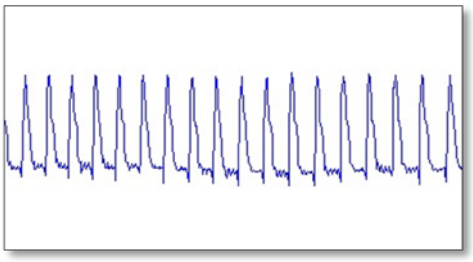

Fig. 2. Ventricular tachycardia.

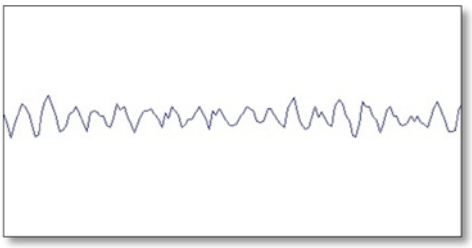

Fig. 3. Ventricular fibrillation.

lead II of electrocardiogram (ECG).

For induction of regional ischemia a surgical needle was passed all round the origin of the left anterior descending coronary artery (LAD), and the ends of the structure were passed through a pipette tip to form a snare. Regional ischemia was induced by tightening the snare and reperfusion was performed by releasing the ends of the structure. After recovery period, 15 minutes was considered as baseline prior to 30 minutes ischemia followed by 60 minutes reperfusion.

Induction of chronic sleep deprivation

The modified multiple platform method (MMPM) was selected to induce chronic sleep deprivation (CSD). Briefly, rats were placed in a water tank (125×44×44 cm.) containing 8 circular platforms, 6.5 cm. in diameter (small platform) for induction of chronic sleep deprivation. Another tank containing 4 circular platforms, 14 cm. in diameter (large platforms) was used for control group. The tank was filled with water until approximately 1 cm. below of platforms top. The rats were allowed to move around freely inside the tank. Muscle atonia due to sleeping led to falling from small platforms the water and awaked the animal. Rats on large platforms could sleep. The large platforms were used for assessing stress due to tank. Food and water were free for using by animals.

Experimental Protocol

The experimental protocol has been shown in figure 1. The hearts were subjected to a stabilization period with krebs-Henseleit buffer perfusion for 20 min. baseline period followed by 30 min. of regional ischemia and 60 min. of reperfusion. All animals were randomly divided into four groups; IR group: rats underwent 30 min. ischemia reperfusion (n=7), CON: control group (n=6), rats has been placed in large multiple platforms for 72h prior to ischemia reperfusion, SD: chronic sleep deprivation group (n=10), 72h sleep deprivation was induced by using small multiple platform prior to ischemia reperfusion. SYM: Sympathectomy group (n=4), 24h before to sleep deprivation, chemical sympathectomy was done by single subcutaneously injection of 6-Hydroxydopamine (100 mg/kg).

Assessment of ventricular arrhythmia

Basis on the Lambeth conventions, ventricular ectopic beats (VEBs) were selected as obvious premature QRS complexes. Ventricular tachycardia (VT) was determined as the occurrence of for or more consecutive VEBs. Ventricular fibrillation (VF) was appeared as unidentifiable and low voltage QRS complexes (Figure 2 and 3).

Ventricular fibrillation may be sustained or may revert spontaneously to a normal sinus rhythm. VF lasting for more than 5 minutes was considered as irreversible.

The severity of arrhythmias was quantified by the following scoring system:

0. 0-50 VEBs with no other arrhythmias over the 25-minute ischemia period,
1. Only 50-500 VEBs,
2. More than 500 VEBs, or one episode of spontaneously reversible VT or VF,
3. 2-30 episodes of spontaneously reversible VT and/or VF
4. More than 30 episodes of spontaneously reversible VT and/or VF
5. Irreversible VF

Statistical analysis

Heart rates were expressed as the Mean±SEM. Tow way ANOVA test was performed for heart rate analyses between groups during baseline, ischemia and reperfusion periods. The arrhythemia scores were analyzed with kruskal-wallis test, and the incidences of VT or VF were compared using the fisher exact test. Significant differences were determined as $p<0.05$.

Results

Heart rate

Heart rate was continuously recorded during the experiments and calculated for 5 minutes at the end of baseline, ischemia and reperfusion. There were no differences between baselines of all groups. Only, heart rate during reperfusion were decreased significantly in IR and CON group ($p<0.05$) as compared with their baselines.

Number of Ventricular tachycardia (VT) episodes during ischemia

In this model of regional ischemia, severe ventricular arrhythmias peaked after 7-15 minutes of left descending artery (LAD) occlusion. Ventricular tachycardia significantly increased in chronic sleep deprivation group as compared with IR group ($p<0.05$, %55 and %15 respectively). Sympathectomy

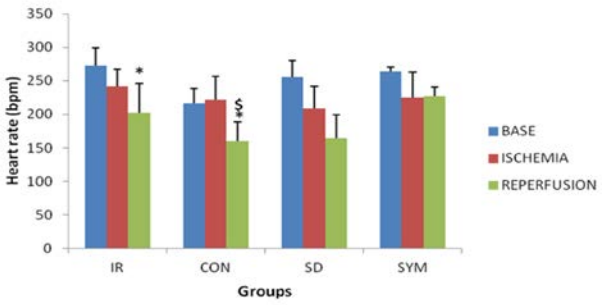

Fig. 4. Heart rate (bpm) in groups. IR: Ischemia reperfusion group, CON: Control group, SD: Sleep deprivation group, SYM: Sympathectomy group.*$p<0.05$ when compared with baseline, $ $p<0.05$ when compared with ischemia.

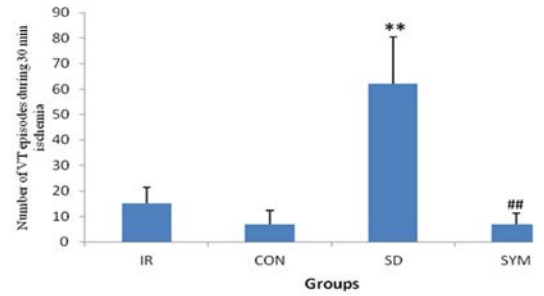

Fig. 5. The number of VT episodes during ischemia period. IR: Ischemia reperfusion group, CON: Control group, SD: Sleep deprivation group, SYM: Sympathectomy group.
**$p<0.01$when compared with IR, ## $p<0.01$ when compared with SD group.

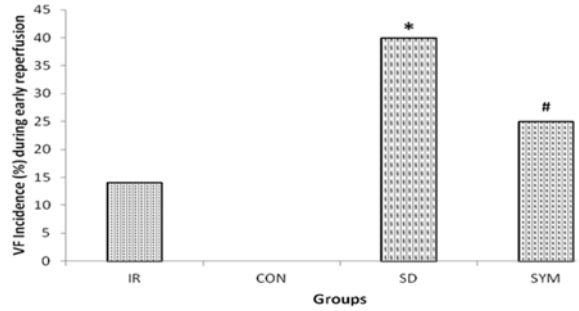

Fig. 6. The incidence of VF during early reperfusion period in groups. IR: Ischemia reperfusion group, CON: Control group, SD: Sleep deprivation group, SYM: Sympathectomy group. *$p<0.05$ as compared with IR group, # $p<0.05$ as compared with SD group.

in SYM group significantly reduced VT incidence when compared with SD group ($p<0.05$, 7% in sympathectomy group). There is no difference in VT incidence between control group (7%) and IR group.

Incidence of Ventricular fibrillation (VF) during reperfusion

The incidence of VF during early reperfusion was significantly increased ($p<0.05$) in sleep deprivation group (40%) as compared with IR group (14%). Sympathectomy significantly could decrease VF incidence (25%) as compared with SD group ($p<0.05$).

Discussion

In this study, we have shown that regional cardiac ischemia reperfusion after 72h chronic sleep deprivation could not change heart rate. The heart rate is a parameter that might play role in arrhythmogenesis. It is considerable that ischemia-induced arrhythmias originate from the boundary zone between the normal region and ischemic area in the boundary zone and shortening of action potential duration (APD) during ischemia will be accompanied by a shortening of refractoriness, which would be pro-arrhythmic (9).

72h of REM sleep deprivation causes to a significant increase in systolic and mean arterial pressure and a relative but non-significant increase in myocardial consumption index, however, it had no effect on the HR of rats (10). Also, it has been shown that 1 week of continuous night shift had no significant effect on the mean heart rate (11). Kato *et al*, reported that a one night of sleep deprivation was related to increased BP and decreased muscle sympathetic nerve activity, but it had no effect on the HR (12). On the other hand, acute sleep deprivation leads to a greater sympathetic influence on the autonomic control of the heart. Endogenous circadian rhythmicity effect on autonomic control of heart rate and the timing of these endogenous rhythms can be altered by extended sleep/rest episodes and associated changes in photoperiod (13).

The present study indicates that the chronic sleep deprivation augmented the number of ventricular tachycardia episodes during ischemia period. It is assumed in ischemic myocardium that catecholamines are involved in exacerbating of arrhythmias by increasing automaticity and stimulation of activity, but it has been shown that there is no association between circulating catecholamines and incidence of arrhythmias in clinical and experimental literatures (9). In our study, chemical sympathectomy could improve ischemia and reperfusion induced ventricular arrhythmias. Moreover, it has been shown that REM sleep deprivation increases PVC (premature ventricular contraction) but not life-threatening ventricular arrhythmias such as VT and VF(10).

Ventricular arrhythmias that result in sudden cardiac death (SCD) are significant unsolved clinical problems. Experimentally, sympathetic stimulation induces changes in ECG repolarization and by decreasing in the threshold, facilitates the initiation of VF. These effects are exaggerated in the presence of cardiac ischemia. The ischemic and infarcted myocardium becomes a substrate exquisitely sensitive to arrhythmia triggers because of regional cellular and tissue remodeling heterogeneity of sympathetic nervous system innervation. Cao et al., reported that patients with a history of ventricular arrhythmias had augmented sympathetic nerve sprouting (mainly in the border of normal myocardium and scar tissues) as compared to patients with similar structural heart disease without arrhythmias (8). Sympathetic nerve sprouting itself can lead to increased incidence of VF without related cardiac ischemia. Increased sympathetic activity, as suggested by heart rate variability analysis, was found to be in the 30 minutes before the onset of ventricular tachyarrhythmia (8). In another study unexpected ventricular tachycardia or ventricular fibrillation have obviously augmented cardiac sympathetic activity compared with appropriate reference groups, based on measurements of the rate of overflow of the sympathetic neurotransmitter, noradrenaline, from the heart to plasma. These clinical findings support a role for cardiac autonomic dysfunction, specifically sympathetic activation and vagal withdrawal, in arrhythmogenesis. Several human studies suggested that sleep loss may effect in elevated catecholamines, increased heart rate and blood pressure, and a shift of sympathovagal stability toward sympathetic dominance. When occurring continually, restricted or disrupted sleep may finally increase the susceptibility

to cardiac electrical instability and coronary heart disease (14). On the other hand, it has been reported that sleep deprivation, ranging between 30h and 72 h, do not make significant changes in response patterns of plasma catecholamines, heart rate, and blood pressure during a subsequent exercise challenge (14). There are several biological interpretations for the increased risk for cardiac heart disease associated with sleep deprivation. Hypertension, increased sympathetic nervous system activity, heart rate, vasoconstriction and salt retention also are side effects of sleep deprivation (10).

Conclusion

Induction of 72h sleep deprivation prior to ischemia and reperfusion increased the probability of VT and VF occurrence during ischemia and reperfusion and chemical sympathectomy could eliminate this effect.

Acknowledgment

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Original Article

Comparison the Efficacy of Pre-Emptive Oral Celecoxib with Acetaminophen in Controlling Post-Operative Pain and Nausea after Lower Limb Surgery under General Anesthesia

Hamid Saryazdi[1], Omid Aghadavoudi[2*], Daryoosh Moradi[1], Amir Hamedani[3]

Abstract

Background: Up to now, there is no single opinion on how to control pain after surgery and molecular and clinical research in this area has been continuing. This study aimed to compare the effect of premedication with oral administration of celecoxib and acetaminophen on postoperative pain relief in the lower extremity surgery under general anesthesia.

Materials and Methods: In a prospective, randomized, double-blinded, clinical trial study, 70 patients undergoing lower limb surgery under general anesthesia were distributed into two equal groups. In the first and second group, oral acetaminophen 1000 mg or celecoxib 400 mg capsules were prescribed one hour before the operation, respectively. Postoperative pain and nausea severity in both groups were evaluated by visual analog scale (VAS) score and compared with each other.

Results: Assessment of pain intensity at 1, 2, 6, 12 and 24 hours after surgery revealed that acetaminophen group at the first hour had more intensity of postoperative pain (5.46±1.17) compared with celecoxib group (4.31±1.32) ($p<0.001$). In rest of the time, there was no significant difference between the two groups. Analysis of variance with repeated observations showed the trend of postoperative pain intensity during the study in both groups had a significant difference ($p=0.013$). The intensity of nausea in the first hour after surgery was significantly more in acetaminophen group compared with celecoxib group (2.8±1.1 vs. 2.2±1.3, $p<0.034$).

Conclusion: Celecoxib may be a better choice in reducing pain and nausea after surgery compared with acetaminophen. Considering no significant adverse effects in many studies, celecoxib may be used as a pre-emptive medication to reduce pain after lower extremity surgery.

Keywords: Premedication, Post-operative, PONV, pain, Celecoxib, Acetaminophen

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Introduction

Pain is one of the common unpleasant postoperative side effects which is intensified by tissue damage and release of histamine and inflammatory mediators during surgery (1). The pain causes increase in sympathetic tone, the production of catabolic hormones like cortisol and catecholamines, anti-diuretic hormone, ACTH, glucagon, renin, angiotensin II and decrease the production of anabolic hormones. The stress response can also create a hypercoagulable state and inhibition of fibrinolysis, increase of plasma viscosity and platelet reactivity. These factors increase the risk of deep-vein thrombosis, vascular graft failure and myocardial infarction (2). Even suppression of inflammatory mediators during surgery could result in lower postoperative pain and morphine requirements (3).

Pre-emptive analgesia reduces pain receptor sensitivity, especially reduces central sensitization to pain (4-6). Opioids for postoperative pain control have side effects such as unbearable itching, nausea and ventilatory suppression. Prostaglandins as a production of tissue damage during surgery are synthesized and causethe sensitivity of the receptors to pain and inflammation (7). Common non-steroidal anti-inflammatory drugs (NSAIDs) as inhibitors of the enzyme cyclooxygenase 1 (COX 1) would prevent the synthesis of these prostaglandins (8). However, a major drawback of these drugs which have restricted their use is inhibition of platelet function and increased risk of postoperative bleeding and gastrointestinal side effects. Enzyme inhibitor cyclooxygenase 2 (COX 2) with fewer side effects may reduce postoperative pain (7). Celecoxib is one of these drugs that its analgesic effects have been proven to produce morphine sparing effect without inducing opioid complications such as respiratory suppression (7, 8). Acetaminophen is an analgesic medication that does not have the side effects of NSAIDs and has been widely used for postoperative pain control (4). According to our literature review, there were few previous studies comparing the preemptive analgesic effect of celecoxib with acetaminophen. Therefore, this study was designed in the patients undergoing lower limb surgery under general anesthesia.

Methods

After approval of Anesthesiology and Critical Care Research Center and obtaining informed consent from patients, this randomized double-blind clinical trial study was performed in 2014. Our study population consisted patients undergoing elective surgery of the lower limbs, aged 18-65 years with American society of anesthesiologists (ASA) physical status I or II. Inclusion criteria included lack of psychiatric problems, coagulopathies, gastrointestinal bleeding, peptic ulcer, chronic pain syndrome, history of seizure, and drug addiction. In any event outside the study protocol resulted in exclusion of the patients.

Based on the formula to estimate sample size to compare averages and by taking 95% confidence level, the power of test as 80%, the standard deviation of postoperative pain score equal to 0.7, the sample size was calculated as 32 patients, but 35 cases were studied in each groups.

Randomization was done by random allocation software and simple random allocation method. The study was designed as blinded in a way that the physician who evaluated the pain and nausea was different from the one who prescribed the premedication.

Oral premedication, included acetaminophen1000mg or celecoxib400 mg in capsules of similar color and shape was done. The drugs were given one hour before surgery with about 100 mL of water. After pre-oxygenation, induction of anesthesia in both groups was performed with the same dose of thiopental sodium (6mg/kg) and atracurium (0.6mg / kg /) and fentanyl (100µgr). Then 1.2% isoflurane + %50 oxygen + %50 N2O was used for maintenance of anesthesia. For analgesia 0.15mg/kg of morphine after induction of anesthesia was used. During anesthesia, pulse oximetry, ECG, blood pressure monitoring, and body temperature monitoring were used and data recorded.

After the operation, in accordance with existing standards, extubation was performed. Extubation time was considered as the duration between the ends of surgery until the time of tracheal extubation. The patients were discharged from recovery room based on modified Aldrete score. The level of consciousness

of the patients at 1, 2, 6, 12 and 24 hours after surgery, according to Ramsay sedation scale (score from 1 to 6), was determined. The intensity of pain was recorded according to visual analog scale (VAS) score at admission to recovery and then 1, 2, 6, 12 and 24 hours after surgery. If the VAS score was 4 or more, intravenous pethidine 0.5mg/kg was administered. Also nausea with a VAS score of more than 4or any episodes of vomiting was treated with intravenous metoclopramide 0.15 mg/kg. All medications and their possible side effects, accompanying with the time of receiving the first analgesic dose were recorded.

Data were analyzed by SPSS software (Version 22.0. Armonk, NY: IBM Corp.). T-test for intergroup comparison of quantitative variables, Chi square test to compare nominal data, and the analysis of variance with repeated observations for trend changes in variables during study was used.

Results

In this study, 70 patients were studied in the two groups of 35 recipients of the acetaminophen and celecoxib during the intervening period. No patient was excluded from the study because of adverse events (Figure 1). In Table 1, the distribution of general demographic characteristics of both groups is shown. The average age, weight, gender, ASA status, duration of surgery, anesthesia and extubation time and recovery duration were not significantly different between the two groups. Hemodynamic parameters in patients during the study period showed no statistically significant difference between the two groups in regard to mean changes in heart rate and blood pressure. In Table 2, severity of pain and nausea according to VAS score is shown. Acetaminophen group had more pain in the first hour after surgery, but in other times, there was no significant difference between the two groups in this regard. Analysis of variance with repeated observations during the study showed that the two groups had significant difference in mean pain intensity trend ($p = 0.013$). The intensity of nausea in the first hour after surgery was significantly more in acetaminophen group compared with celecoxib group. Vomiting episodes during the postoperative period are shown in Figure 2 and according to Fisher's exact test; the difference between the two groups was not significant ($p>0.05$).

Average time to initial analgesic requirement in the postoperative period was 1.74±1.4 and 2.49±2.7 hours in acetaminophen and celecoxib groups, respectively ($p=0.16$). The mean total analgesic drug prescribed during the postoperative period in acetaminophen and celecoxib groups was

Table 1: Distribution of demographic characteristics in the two groups.

Group Variable		Acetaminophen	Celecoxib	P value
Age (year)		28.6 ± 5.2	29.7 ± 5.5	0.4
Weight (kg)		68.4 ± 9.7	69.9 ± 8	0.47
Sex	Male	28 (80)	32(91.4)	0.17
Number (%)	Female	7 (20)	3 (8.6)	
ASA	I	32 (91.4)	29 (82.9)	0.28
	II	3 (8.6)	6 (17.1)	
Operation time (min)		107 ± 28.4	115.4 ± 27	0.21
Anesthesia time (min)		122.9 ± 27.4	128.6 ± 25.9	0.21
Recovery time (min)		94.86± 15.2	91.42± 14.4	0.34
Extubation time (min)		6.09± 1.04	6.37± 1.08	0.27

Table 2: Mean±SD of postoperative pain and nausea intensity in both groups.

Group / Time	Postoperative pain intensity			Postoperative nausea intensity		
	Acetaminophen	**Celecoxib**	**P value***	**Acetaminophen**	**Celecoxib**	**P value***
1st hour	5.5 ± 1.2	4.3 ± 1.3	0.001	2.8 ± 1.1	2.2 ± 1.3	0.034
2nd hour	5.9 ± 1.3	5.4 ± 1.4	0.1	2.5 ± 1.1	2.2 ± 1.7	0.4
6th hour	4.6 ± 1.2	4.2 ± 1.3	0.14	1.5 ± 0.9	1.5 ± 1.5	0.92
12th hour	4 ± 1.2	3.5 ± 1.6	0.12	1 ± 0.9	1.1± 1.2	0.74
24th hour	2.6 ± 1.2	2.7 ± 1.3	0.85	0.2 ± 0.4	0.8±0.9	0.002
P value**	0.013			0.81		

* The difference between the two groups at any time by t-test analysis
** The difference between the two groups change according to analysis of variance with repeated views

Table 3: Dosage (mean±SD) of rescue drug administration (mg) for control of postoperative pain (acetaminophen) and nausea (metoclopramide) in the two groups.

Group / Time	Analgesic			Metoclopramide		
	Acetaminophen	**Celecoxib**	**P value***	**Acetaminophen**	**Celecoxib**	**P value***
1st hour	28.7 ± 9.2	26.7 ± 6.5	0.46	10 ± 0	10 ± 0	1
2nd hour	32 ± 16.8	30 ± 10.2	0.62	10 ± 0	10 ± 0	1
6th hour	25 ± 0	26.6±6.2	0.4	0	10 ± 0	1
12th hour	25 ± 0	25 ± 0	1	0	0	1
24th hour	25 ± 0	25 ± 0	1	0	0	1
P value**	0.51			0.99		

*the difference between the two groups at any time by t-test analysis
**the difference between the two groups changes according to analysis of variance with repeated views

58.57±27.1 mg and 54.29±23.9 mg, respectively (p=0.49) (Table 3).

Discussion

The primary objective of this study was to compare the effect of pre-emptive oral administration

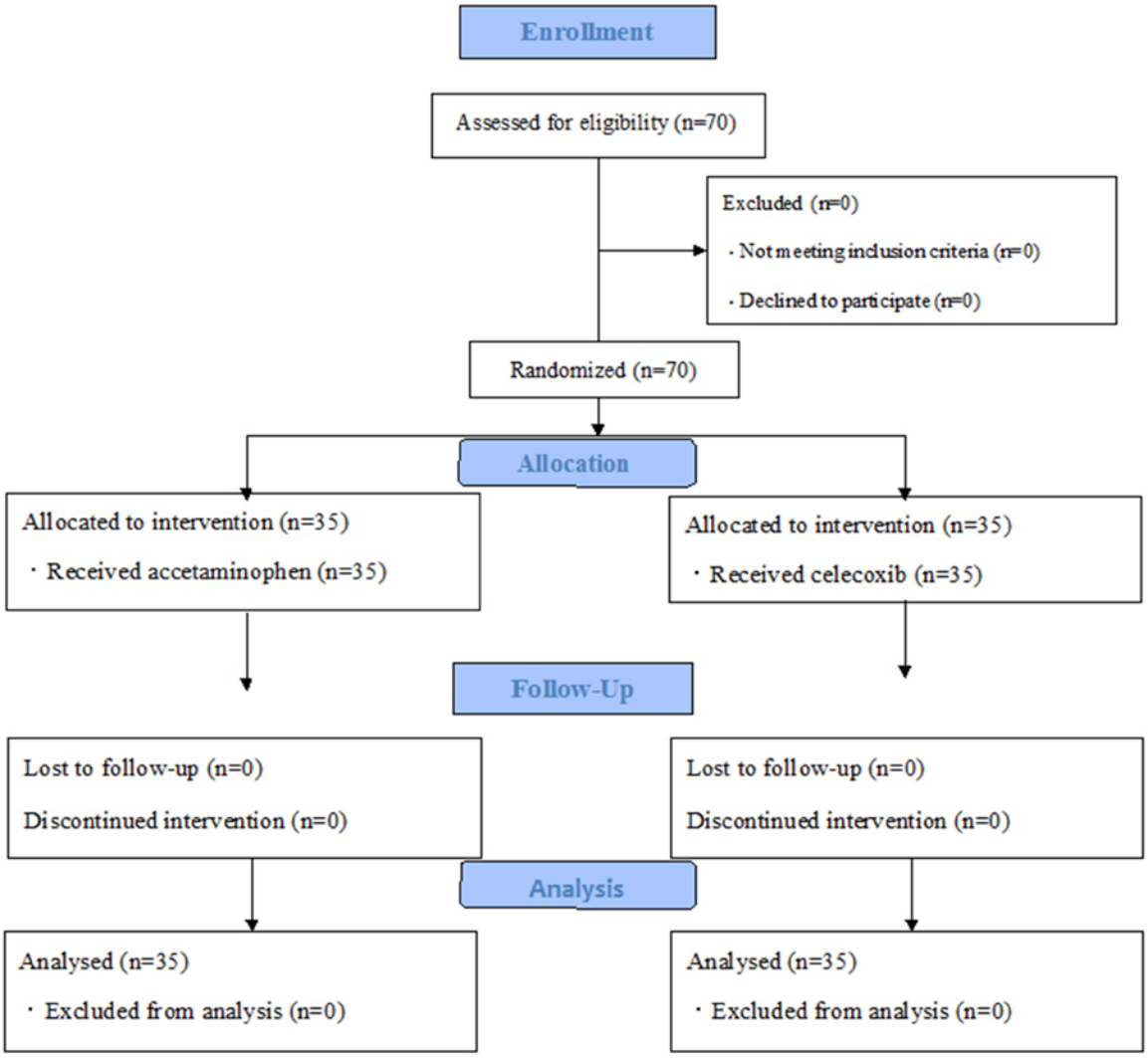

Fig. 1. CONSORT Flow Diagram.

of celecoxib and acetaminophen for pain relief after lower limb surgery under general anesthesia. The secondary outcome of the study was to measure rescue analgesic consumption and duration of recovery stay, and occurrence of PONV. In this study celecoxib and acetaminophen did not have any adverse effects on patient's hemodynamic parameters, and in this sense, the use of both drugs is safe. Celecoxib compared to acetaminophen, particularly at one hour after surgery, was associated with a further reduction in patients' pain and nausea and less rescue analgesic drug requirements. However, considering other times of measurement and the total dose of rescue analgesic and average time to initial analgesic requirement, it could be concluded that both drugs can be administered in preoperative period. In this study placebo group was not considered because previous studies had shown that acetaminophen or celecoxib has more efficiency than placebo in controlling perioperative pain.

Mardani-Kivi et al., showed that pre-emptive administration of celecoxib, 2 hours before knee arthroscopic surgery, resulted in significant reduction in pain intensity and opioid consumption at 6 and 24h post operation, but side effects of analgesics such as nausea and vomiting, sedation, and dizziness were not significantly different from the placebo group (9). In our study acetaminophen was used instead of placebo one hour before surgery. In another study conducted by Zhang et al., pre-emptive administration of 200 mg

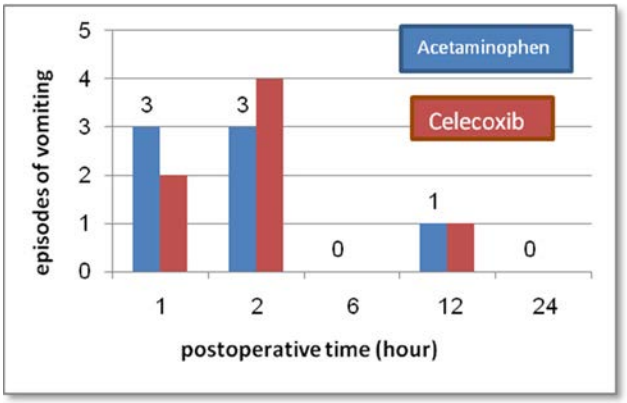

Fig. 2. The frequency of vomiting episodes after surgery in both groups (p>0.05, according to Fisher's exact test).

celecoxib, one hour before arthroscopic hip surgery under spinal anesthesia, resulted in less pain and narcotic consumption at 12 and 24 h postoperatively, but the VAS score of pain was not different from the placebo group in the recovery room (10).

In one study by Fenlon et al., oral vs. intravenous paracetamol for lower third molar extractions under general anesthesia was compared. They concluded that single dose oral paracetamol given 45 min before surgery was not inferior to intravenous preparations given after induction of anesthesia in controlling post-operative pain (11). Therefore, oral premedication can be administered for post-operative pain control as in our study. In one study by Alimian *et al*, patients scheduled for elective laparotomy, intravenous paracetamol or morphine was administered by infusion pump after surgery. They concluded that paracetamol is insufficient for postoperative pain control in the first eight hour postoperatively (12). This is in accordance with our study. However, the effect of central pain pathways involved in abdominal surgeries and the efficacy of oral medications such as paracetamol or celecoxib may be assessed in future studies. In two other independent studies, the effect of celecoxib and acetaminophen for pain relief after surgery has been investigated separately.

Lin et al., in a systematic review showed that perioperative administration of COX-2 selective inhibitors resulted in less pain scores, opioid consumption, itching and PONV and better active range of motion in patients undergoing total knee arthroplasty. Meanwhile, the volume of blood loss was not significantly increased post-operatively (13). Also in our study the patients who received celecoxib, suffered less from PONV and this is another benefit of using COX-2 selective inhibitors in preoperative period. Khalili and colleagues have shown that prescription of preemptive and preventive intravenous acetaminophen, in patients under spinal anesthesia, reduces pain intensity and additional analgesic consumption in the first 24 hours after lower limb surgery (14). In their study acetaminophen was compared to placebo, but in our study acetaminophen compared to celecoxib was less efficient. Kashefi and colleagues showed that oral administration of celecoxib 200mg compared to 320mg of acetaminophen, administered 2 hours before surgery significantly reduced pain in the first 4 hours after surgery (15). They used lower doses of celecoxib and more vomiting episodes may be related to this lower dose comparison with our study.

Some limitations of our study were: Postoperative pain severity after the first hour and total rescue analgesic requirements did not differed in both groups. It may be related to low sample size or time of premedication. Therefore, performing studies with larger sample sizes are recommended. In our study we did not measure the real need for intraoperative analgesic requirement and all patients received a fix dose of intraoperative morphine. It is recommended that in future studies, this point will be considered. Also comparing biomarkers of pain may be considered, as pain diagnosis and management need more objective markers than VAS score or monitoring changes in the autonomic nervous system.

Conclusion

Considering the results of the present study and comparison with other studies, it seems that celecoxib has better efficacy in reducing post-operative pain and nausea than acetaminophen. Given the indications of drug and the medical advice, celecoxib can be used as a drug for pain and nausea relief after surgery.

Acknowledgment

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Case Report

Identification of the First Iranian Family with "γArg275Cys" Mutation (Fibrinogen Tokyo II)

Gholamreza Toogeh[1], Maryam Helali[2], Shaban Alizadeh[2*], Akbar Dorgalaleh[3]

Abstract

Background: Inherited fibrinogen deficiencies are classified into two categories: quantitative (including a fibrinogenemia and hypofibrinogenemia) and qualitative (including dysfibrinogenemia). Any mutation in fibrinogen genes accounts for one of these disorders.

Case Report: This article reports an Iranian family with dysfibrinogenemia without any clinical signs accidentally diagnosed by routine coagulation tests with slightly elevated PT and APTT a few years ago. For determination of the disease which causing genetic aberration in fibrinogen genes, DNA sequencing of three hot spots of these genes (i.e. exon 2 of FGA, exon 2 of FGB and exon 8 of FGG) was performed. Analysis of sequencing results revealed a heterozygous missense mutation c.901 C>T (Arg275Cys) in exon 8 of FGG in mother and children. No mutation was detected in father's sample. Fibrinogen with this mutation is known as Tokyo II.

Conclusion: γArg275Cys is a heterozygous mutation that impairs the function of fibrinogen and has been solely reported in dysfibrinogenemic patients. Clinical findings in this family (no history of bleeding and thrombosis) were compatible with molecular results, because fibrinogen Tokyo II does not have a thrombotic or hemorrhagic nature and lack of clinical signs in this family is not unexpected.

Keywords: Fibrinogen, Tokyo II, Dysfibrinogenemia

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Introduction

In hepatocytes, transcription of three clustered genes of FGA, FGB and FGG leads to production of three polypeptide chains Aα, Bβ, and γ, eventually causes fibrinogen formation. This factor is secreted to blood with normal plasma level of 1.5-3.5 gr/L (1). Inherited fibrinogen deficiencies are rare bleeding disorders (RBDs) that classified into two categories: quantitative, including a fibrinogenemia (factor level <0.1 gr/L) and hypofibrinogenemia (factor level 0.5-1.5 gr/L) as well as qualitative, including dysfibrinogenemia that is characterized with abnormal function of fibrinogen (2). The main goal of coagulation cascade is conversion of fibrinogen to fibrin polymers and clot formation (1). Mutation in one of the fibrinogen genes can account for any of these disorders. The most important causative mutations of congenital dysfibrinogenemia (CD) have been identified in exon 2 of FGA, exon 2 of FGB and exon 8 of FGG (3). Here we described the first

dysfibrinogenemic Iranian family with γArg275Cys mutation bearing fibrinogen Tokyo II which was not reported previously in Kermanshah Province.

Case Presentation

This study was performed in 2015 and reports a family with consanguineous marriage from Kermanshah Province, Sahne city of Iran. Mother of case was diagnosed as dysfibrinogenemic just during routine coagulation tests before caesarian section 16 years ago. Prolonged prothrombin time (PT) and slightly elevated activated partial thromboplastin time (APTT) along, with abnormal fibrinogen activity measured by Clause method were indicative of dysfibrinogenemia. Moreover, lack of liver failure symptoms like increased plasma liver enzymes excluded acquired form of the disorder. Two sons were screened for disease when they were one year old and CD was confirmed in them. Table 1 displays the results of coagulation tests in this family. As it is evident in Table 1, the father was apparently healthy. Considering the clinical manifestations, none of the members of this family had experienced bleeding (such as menorrhagia and postpartum hemorrhage in mother, epistaxis, oral cavity bleeding, bleeding after dental extraction and hematomas) or thrombosis.

This study was continued by molecular tests for determination of disease causing genetic aberration in fibrinogen genes. After receiving the consent form and sampling, DNA was extracted from whole blood of all family members using DNA extraction kit (Blood & Tissue Genomic DNA Extraction Miniprep System; Viogene, Taipei, Taiwan). After that, DNA was used for amplification of hot spots CD variation according to updated fibrinogen mutation GEHT web site (www.geht.org/databaseang/fibrinogen), including exon 2 of FGA, exon 2 of FGB and exon 8 of FGG. The primers used for PCR amplification and sequencing of these three exons as well as PCR information are shown in Table 2. After purification of PCR products by agarose gel extraction, they were cycle sequenced by forward and reverse primers.

Table 1: Coagulation test results in all the family members.

Members	PT(sec)	APTT (sec)	FC* (functional assay) (gr/L)
Father	12	30.5	2.6
Mother	17	38	0.45
Son 1	17.6	38.3	0.47
Son 2	16.7	40.6	0.6

Prothrombin Time/Normal range: 11.8 -14.6 second
Activated Partial Thromboplastin Time/Normal range: 30-38 second. * Fibrinogen concentration.Normal range: 1.5-3.5 gr/L

Interpretation of cycle sequencing results by Mutation Surveyor version 3.3 and Chromas version 2.4.3 software revealed a heterozygous substitution of C>T at position 901 of cDNA (c.901 C>T) in exon 8 of FGG, which leads to replacement of arginine by cysteine at position 275 of mature gamma chain (position 301 in immature form). This missense mutation is a known disease causing variant "rs12913087" for CD. The fibrinogen bearing this mutation is named *Tokyo II* (Other names: Osaka 2 /Tochigi 1 /Moricka 1). As expected, this variation was not found in the sample taken from healthy father as expected (Figure 1).

Discussion

We have reported the first Iranian family with fibrinogen Tokyo II. Despite the high prevalence of fibrinogen disorders in Iran in comparison to other countries, molecular investigation of this disorder is uncommon in this province.

According to World Federation of Hemophilia (WFH) in 2013, Iran has highest number of patients with inherited fibrinogen disorders (5). Because of the presence of some cases with asymptomatic dysfibrinogenemia (like this family), a larger number of patients is estimated in communities.

Arg275Cys mutation involves a C-terminal region of gamma chain, which is crucial for end to end alignment at fibrin monomers. So, this amino acid exchange is thought to be associated with abnormal interaction in fibrin monomers (6).

In comparison to known thrombotic mutation Arg573Cys in alpha chain of fibrinogen in CD patients, Arg275Cys in gamma chain does not involve

Table 2: Primers used for amplification and sequencing.

Fibrinogen gene	Forward	Reverse	Annealing temperature (°C)	Amplicon size (bp)
FGA exon 2	TGAGAGTGCCATCTC TTCCTG	AAATCCTGTCTGTTCACC CACT (4)	58	440
FGB exon 2	GAGGGTGTTGGAATA GTTACA	ACAGGCTTTCTCTGCATG AG	53	320
FGG exon 8	TTCCAAGGAAGCATC CTAC	GTCTAAAGGAGATCCCA CAAC	55.5	657

thrombotic and bleeding effects (7).

Two mutations of Arg275Cys and Arg275His were identified in 17% of dysfibrinogenemic patients from UK (8). Also, more comprehensive study at Finland on 101 affected subjects reported that Arg275Cys mutation is the most common cause of dysfibrinogenemia with prevalence of 32.7% (9). Nevertheless, another study has indicated Arg16Cys mutation in alpha chain as the main variation of hot spot in dysfibrinogenemia (10). So, mutations detected incase reports or case series with dysfibrinogenemia can provide more data for resolution of these conflicts.

The presence of two affected sons in a family with healthy father and carrier mother (heterozygous mutation) may be justified by autosomal dominant inheritance pattern of CD. So far, almost all of the mutations detected in CD subjects have been heterozygous.

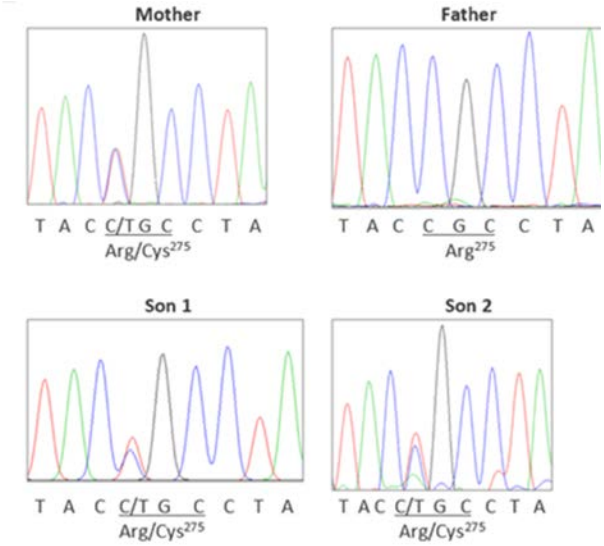

Fig. 1. Detection of Arg275Cys mutation of γ-fibrinogen in three affected members. Heterozygous mutation was shown in codon 901(CGC to TGC) in mother and her affected sons.

Conclusion

Arg275Cys is a heterozygous mutation that impairs the function of fibrinogen and has been solely reported in dysfibrinogenemic patients. Clinical findings in this family (no history of bleeding and thrombosis) were compatible with molecular results since Tokyo II fibrinogen does not have thrombotic and hemorrhagic nature and lack of clinical signs in this family is not unexpected.

Acknowledgment

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Conflicts of Interest

The authors declare that they have no conflict

of interest.

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Brief Communication

Anesthetic Consideration of Niemann-Pick Disease Type C

Masoud Nashibi[1], Ardeshir Tajbakhsh[1], Farhad Safari[1], Kamran Mottaghi[1*]

Abstract

Niemann-Pick disease type C (NPC) is a rare, autosomal recessive, neurometabolic disorder associated with the accumulation of unesterified cholesterol in lysosomes and late endosomes. Because of multiple organ involvement and wide range of clinical manifestations, these patients will demand multiple diagnostic and therapeutic procedures requiring anesthesia. Since pathogenesis of this disease is still unknown and further investigations on cellular and molecular basis of NPC is needed. In this report we present a known case of NPC1 requiring anesthesia for Percutaneous Endoscopic Gastrostomy and a brief review about molecular basis and recent advances in this field.

Keywords: Anesthesia, Niemann-Pick Disease, Gene Mutation (NPC1); Intracellular Cholesterol Transport

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Introduction

Albert Niemann and Ludwig Pick in 1920's introduced an autosomal recessive disease characterized by disorders in liposomal lipid and sphyngomyeline storage, by common feature of hepatosplenomegaly with or without neurological involvements. Later in 1958 Crocker proposed a four group classification (A-D) based on age of onset, clinical implications and level of sphingomyelin storage in tissues (1, 2).

Type A defined by early CNS involvements and massive storage of visceral and cerebral sphingomyelin. Type B has a chronic course with visceral involvement and sparing CNS. Type C and D defined by sub-acute CNS involvement with milder visceral storage. Type D patients were individualized essentially on their homogenous Nova Scotia Acadian origin (1, 3).

Niemann-Pick Type C (NP-C), with a prevalence of 1/100000 – 1/120000, is characterized by progressive, disabling neurologic features (cerebellar ataxia, dysarthria, dysphagia, progressive dementia, cataplexy, seizures, dystonia and supranuclear gaze palsy) with mild visceral storages (liver, spleen, lungs) (1, 3). It is caused by mutations in either of two genes mentioned as NPC1 and NPC2.

NPC1 is involved in 95% of patients, including type D while NPC2 is present in certain families. These genes participate in cellular post lysosomal/late endosomal transport of cholesterol (4, 5). These changes result in sequestration of unesterified cholesterol in lysosomes and late endosomes (1, 2). Onset of NPC ranges from perinatal period till seventh decade of life, therefore life expectancy range from few days (Fetal hydrops) till over 60s; though majority of them die between 10 and 25 years from neurologic manifestations (1, 2).

Diagnosis of this disease is based on multidisciplinary process involving clinical assessments, histological, electron microscopic tests,

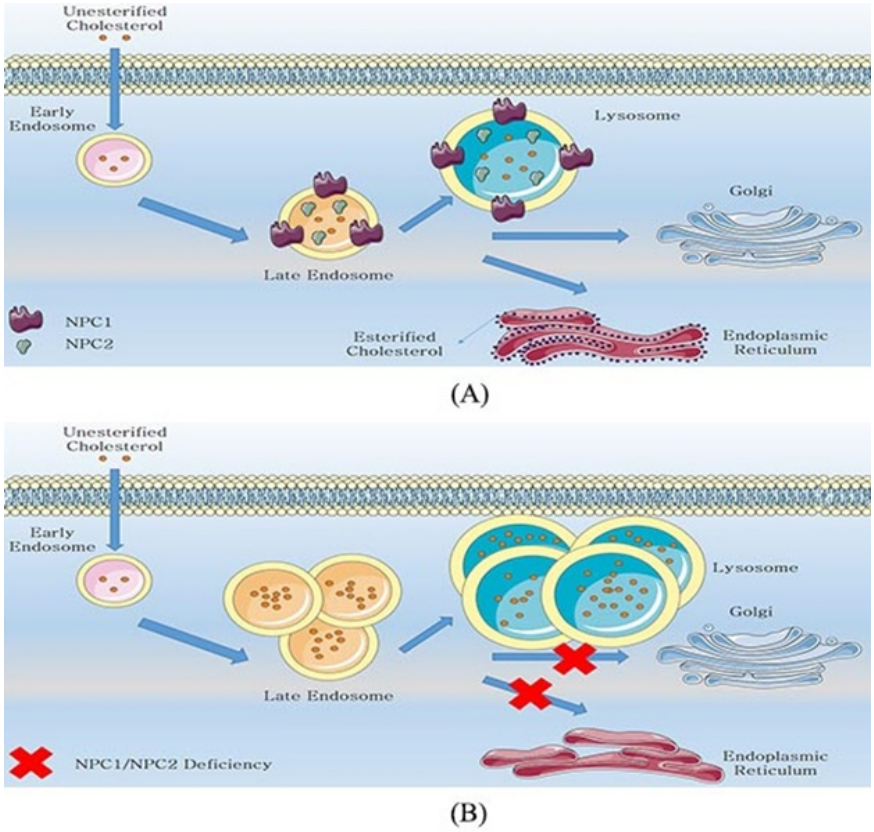

Fig. 1. (A) Normal esterification of cholesterol; (B) Esterification of cholesterol in NPC1/2**.**

biochemical, and molecular genetics laboratory studies (2). Final confirmation is based on demonstration of characteristics intralyposomal accumulation of unesterified cholesterol (Filipin staining in skin fibroblasts) and/or the identification of mutations in either the NPC1 or NPC2 genes (2). Novice areas of diagnosis are plasma oxysterols (particularly cholestane-3β,5α,6β-triol and 7-ketocholesterol), certain sphingolipids such as lysosphingosine (2).

Major clinical manifestations are dysphagia, recurrent aspirations leading to gastrostomy, severe epilepsy, severe hepatosplenomegaly and dramatic psychiatric disorders (1, 3, 6).

The only available approved therapy is Miglustat which could be prescribed as neurologic symptoms appeared. This drug can slower the progression of neurologic damage and also have some positive impacts on developing and progression of dysphagia (4, 6 7).

As discussed, the nature of the disease made it a target for diagnostic and therapeutic procedures that require anesthesia. This report will address anesthesia management of Niemann-Pick C and discuss cellular and molecular advances in this field.

Brief Report

A 9 years old boy, known case of Niemann-Pick C (NPC), was admitted to our hospital due to uncontrolled seizures and respiratory distress.

He was born at full term to nonconsanguineous parents by normal vaginal delivery without remarkable family history. His growth was normal in his first 6 months. Later on, splenomegaly observed and neurodevelopmental milestones were lost. Further examinations confirmed the diagnosis of NPC by cutaneous biopsy and Filipin staining. Since then Meglustat 100 mg TDS was initiated. He had developmental progression for the next 6 years till neurologic symptoms including seizures, ataxia, incontinency, and dysphagia emerged. His weekly seizures were managed by Lamotrigine, Sodium valproate and Prednisolone. Unfortunately respiratory symptoms (Productive cough, Wheezing) occurs from former month due to uncontrolled seizures, dysphagia

Table 1: Anesthetic consideration of Niemann-Pick Disease; *TV*, tidal volume, *RR*, respiratory rate, *FRC*, functional residual capacity, *TIVA*, total intravenous anesthesia.

Organ System	Associated Comorbidities	Anesthetic Consideration
Respiratory System	Restrictive Lung Disease	Lower TV and higher RR
	Recurrent Aspiration Pneumonia	
Gastrointestinal System	Hepatosplenomegaly	Decreased FRC and Increase in episodes of desaturation
	Ascites	
	Liver Failure	Consider Dosage Adjustments
	Sialorrhea	Anticholinergic as premedication could be beneficial
Airway	Difficult intubation	Consider anticipated difficult intubation algorithm
Hematologic System	Thrombocytopenia	Increased risk of bleeding
Central Nervous System	Seizure	Continue antiepileptic agents and avoid epileptogenic drugs
		Use of TIVA

and severe sialorrhea, suggesting aspirating pneumonia.

During his admission, antibiotics were prescribed. Antiepileptics changed to Topiramate and Levetiracetam. He was feeding through NG tube. After alleviation of respiratory symptoms and control of seizures, he was transferred for Percutaneous Endoscopic Gastrostomy.

On preoperative assessment, the patient weighed 40 Kg, the physical examination revealed bilateral rhonchi with increase in respiratory work. Baseline SpO2 was 94% in room air. Laboratory Data was within normal range.

Atropine 0.8mg/IV, Midazolam 1mg/IV and Fentanyl 50μg/IV was prescribed as premedication in endoscopic ward under full monitoring (ECG, Pulse oximetry, and NIBP). Induction was achieved by Propofol 80 mg/IV and the patient was intubated with ETT #5.0 under Direct Laryngoscopy. Infusion of Propofol was started for maintenance at 250 mg/hr. During procedure vital sign was stable and about 20 minutes after secession of maintenance the patient was extubated and transferred to ward.

Discussion

NPC is caused by mutations in one of the two genes called NPC1 or NPC2. 95% of cases have NPC1 mutation which encodes a large glycoprotein in late endosomal location. This gene mapped to chromosome 18q11-q12, spans 56 kbp and contains 25 exons. NPC2 encodes a small soluble lysosomal protein which binds cholesterol with high affinity (8). NPC2 mapped to chromosome 14q24.3, spans 13.5 Kbp and contains 5 exons. Deficiency in both types causes impairment in processing and utilization of endocytosed cholesterol (1, 8). The cellular hall mark of this disease is inability of cholesterol to transport from late endosomes to plasma membrane or reticulum endoplasmic therefore accumulation of cholesterol and products will occur (Figure 1) (1).

Therefore cholesterol storage is impeded and resulted in different pattern of accumulation in neuronal and extra-neuronal tissues. These changes cause sphingomyelin metabolism alteration in extra neuronal tissues. Unesterified cholesterol, sphingomyelin, bisphosphate, glycolipids, and free sphingosine and sphinganine will accumulate in liver and spleen(1). In neurons, accumulation of Glycosphingolipids including GM2 and GM3 gangliosides occurs and cause meganeurite formation, growth of ectopic dendrites, neurofibrillary tangles formation, neuroinflammation, and neuroaxonal dystrophy (8). On the other hand, some proteins like Rab9 or mannose-6-phosphate receptors transfer to cell membrane by late lysosomal system. Cholesterol accumulation could also impair this trafficking (1). Unexplainably neural death occurs mostly in Purkinje cells of the cerebellum. The function of these proteins are not defined yet so the exact mechanism and pathophysiology remains a mystery (1).

The only approved therapy for NPC so far is Miglustat (N-butyl-deoxynojirimycin). It is an iminosuger inhibitor of glucosylceramide synthase (1). This drug can stabilize the neurological manifestations including dysphagia (6). Miglustat by inhibiting glucosylceramide synthase reduce the synthesis of glucosylceramide-based glycosphingolipid in CNS (6). But long term clinical outcomes are still unclear (6). Other drugs used for symptom therapy including antiepileptic drugs (treatment of seizure), clomipramine, protriptyline, or modafinil (treatment of Cataplexy), anticholinergic agents (treatment of dystonia and tremor), Melotonin (treatment of insomnia). Physiotherapy for management of muscle spasticity and contracture is useful. As the major cause of mortality in these patients is aspiration pneumonia, the most important part of managing this disease is handling feeding abnormalities (1, 6). Current researches in the field of treating this disease is based on animal models mostly transgenic mice and cats and also testing various compounds like imatinib, curcumin, NSAIDs, neurosteroids (allopregnolone) and 2-HP-ß-cyclodextrin. The last compound showed significant improvement in disease natural history in animal models but needs further investigations (1).

The exact function of NPC genes are unknown therefore the pathophysiology of this disease is still a mystery (4). So the target metabolite in brain causing the neuroinflammatory responses remains unknown. These results are in lack of a biochemical blood test for evaluating prognosis or diagnosis (1, 4). Currently the gold standard for diagnosis is skin biopsy however recent advances suggest oxysterol profile as a biomarker for NPC. Although more researches are needed to define it as an indicator, these findings could change the future treatment and research developments (1, 4).

As mentioned above, NPC is a disease which involves multiple organs including central nervous system, respiratory system and gastrointestinal system (hepatosplenomegaly). Therefore this disease could interfere with routine anesthetic plans (3, 5). Restrictive lung disease arise from recurrent aspirations demand specific consideration in ventilator setup, so lower tidal volumes and increase in respiratory rate could be helpful for these patients. Another problem is the liver damage caused by storage of lipids, which needs careful selection of anesthetic drugs (5). Also hepatomegaly could accompany ascites which leads to decreased Functional Residual Capacity (3). there are some reports indicating the association between NPC and difficult airway therefore considering options for intubation would be wise (1). on the other hand, thrombocytopenia accompanied by this disease, could potentiate the risk of bleeding (1). Due to vigorous

secretions anticholinergic drugs prior to anesthesia could improve the outcome of the procedure (3). It is demonstrated that hyperventilation plus high concentrations of Sevoflurane could induce seizures. Therefore to avoid this phenomenon continuing antiepileptic agents in perioperative period and use of TIVA instead of volatiles is recommended (3). The technique of choice is the one in which satisfactory condition for procedure is established rapidly and safely and also the recovery should be safe and predicted with minimal sequel for the patient (5).

Conclusion

Although almost a century has passed since the introduction of NPC, there are many unknown aspects which could be a good target for future researches. These unrevealed areas of investigations include absence of biomarker for evaluating treatments, exact pathophysiology, and introduction of effective treatment. Diversity of clinical manifestations together with unknown pathophysiology results in the complexity and enigmatic features of this disease.

Some of manifestations of NPC can affect the anesthetic plan. These comorbidities are summarized in table 1. In conclusion by considering comorbidities and anesthetic implications mentioned above and severity and type of procedure or surgery, each patient with different clinical symptoms could benefit from different anesthetic approaches (1, 5).

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

Opium Abuse and its Problems in Anesthesia Practice: a Review from Bench to Bedside

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Abstract

Opium is a derivative of opium poppy; the species of plant which its extract is used for preparing opium. Opium abuse is considered under drug dependency classification of psychiatric diseases and opium abusers have a number of major challenges before, during and after anesthesia for surgical operations (i.e. the perioperative period). This article reviews these clinical challenges during the perioperative period to discuss the new clinical findings for these patients and to demonstrate some of the main problems that physicians are encountered.

Keywords: opium, abuse, anesthesia

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Introduction

Opium is one of the oldest substances being abused worldwide. It has been mentioned as an anesthetic medication many years ago, including the citations to opioid by Shahnameh and Avicenna (1-5). However, in the modern medical practice, opioid derivatives are used just as their pharmaceutical compounds and those patients abusing opium impose a great challenge for the medical team especially during the course of an operation (3, 6-9). In this review, we have focused on the problems related to the clinical management of opium abuser patients during anesthesia based on the available evidence in this field.

Evidence Acquisition

The data of this study was gained through a systematic search in PubMed. The search strategy included the following keywords: opium, anesthesia, and anesthesiology. This search was limited to the last 10 years i.e. from 2005 to 2015. For "(opium) AND (anesthesia)", the search resulted in 35 items while for "(opium) AND (anesthesiology)" the search resulted in 31 manuscripts. Then, the overlaps between the two search results were cleared and the final number of manuscripts without being repeated in search query was 50. Throughout the study, we found 40 complementary articles which were in direct relation during the search to the primary 45 articles; so they were added to our primary sample and finally we had 95 articles related to "(opium) OR (anesthesia)" or "(opium) OR (anesthesiology)".

Discussion

Based on the available evidence, we categorized the current studies. For this purpose, we will discuss:

1. **Basic studies** related to problems of opium abuser in anesthesia; which is primarily involved with those researches in basic science

1-1- Genetic factors in pain management for opium abusers

1-2- Receptor response in pain management for opium abusers

1-3- Immunologic mechanisms for pain management in opium abusers

1-4- Anatomic classification for pain control in opium abuser

2. Then, we have described the **clinical challenges** of opium abusers in anesthesia; so, we have used the time course of patient management during a surgical operation as the basis for the clinical classification:

2-1- preoperative period: before the operation

2-2- intraoperative period: at the time of the surgical procedure

2-3- postoperative period: after surgery

1- Basic studies related to problems of opium abuser in anesthesia

These studies explain how chronic opium abuse causes a number of cellular changes in pain perception. First we discuss mechanism based changes, then, we consider 3 anatomic sources for pain control: brain, spinal cord and peripheral nerves. Although these studies have been classified here in a number of sub-classes, often these classifications have overlaps.

1-1- Genetic factors in pain management for opium abusers:

there are a number of genes that predispose people to opium abuse; these genes are the focus for further studies; we should believe in "genetic predisposition" when treating opium abusers during the perioperative period. Also, manipulating these genes could be a potential, but very effective method for changing the behavioral patterns of opium abusers. Possibly, in near future, we could at least detect these genes to treat opium abusers undergoing anesthesia in a more appropriate manner or even, we will be able to produce much more appropriate pharmacologic agents for managing opium abusers undergoing anesthesia (10-12)

1-2- Receptor response in pain management for opium abusers:

It is including the changes in neural receptors that have occurred in opium abusers. These changes affect the pain receptors at the cellular and sub-cellular level. In other words, the receptors of opium have changed their response from a normal response to pain to an abnormal response. These changes have a number of mechanisms, resulting from the reaction of receptors to repeated opium exposure; i.e. opium attachment to the receptor does not elicit the normal intracellular RNA production process; which would result in synthesis of different and abnormal proteins; the resulting change in protein synthesis causes different clinical responses like pain intolerance, hyperalgesia, allodynia or other clinical phenomena seen in these patients which are primarily due to modifications in receptor response; these mechanisms are under further research and contribute an active field of studies which could create new horizons not only for opium abusers but also for other chronic pain patients and include: up-regulation of substance P, up-regulation of Calcium Gene Related Peptide (CGRP), changes in inhibition of Nitric Oxide, modifications in inhibitors of cyclooxygenase, abnormal inhibition of Protein kinase C, modifications in antagonistic response of NMDA (N-methyl-D-aspartate) receptor, changes in antagonism of alpha-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid (AMPA) receptor, antagonism of cholecystokinin (CCK), changes in L-type Calcium channel response (L-type Ca channel could be blocked with amlodipine to overcome some effects of opium tolerance) (13-17).

1-3- Immunologic mechanisms for pain management in opium abusers:

It includes the interactions of the immune system due to repeated opium exposure. In opium abusers, there are a number of well demonstrated changes in immunologic mediators. Immunologic cells and other components of the immunologic system can induce pain intolerance. Some major immunologic responses in opium abusers include: increased ratio of pro-inflammatory interleukins compared to anti-inflammatory interleukins, expression of NK-1 receptor in the dorsal horn of the spine, facilitating pain conduction, the role of Toll-like receptors especially TLR-5 in chronic pain and its modulation and other components of both innate and adaptive immunology system.

These changes will results in partial ineffectiveness of anesthetic agents, needing extra-normal anesthetic drugs or inability to control stress response which its control is an important goal in anesthesia care during perioperative period (18-21).

1-4- Anatomic classification for pain control in opium abusers**:**

It includes brain, spinal cord and peripheral nerves.

Brain related mechanisms: these are due to changed response of brain to neurotransmitters, cellular signal transduction, signal processing and other neural circuit changes in the brain leading at times to partially permanent trophic changes that result in really great challenges in the clinical field. These changes create an abnormal pattern of signal transmission in different parts of brain including thalamus, locus coeruleus and other nuclei. For example: changes in cholecystokinin level in the rostral ventromedial medulla in repeated exposure to opioids results in up-regulation of CCK; increased CCK will activate facilitation of descending pain pathways, which is relayed via the dorsolateral funiculus, leading to hyperalgesia (13, 17, 22-24), the neurons located in locus coeruleus have specific relation with mu receptor and they are coupled with K channels; while, increased excitatory neurotransmission through nucleus paragigantocellularis could be among the mechanisms that make opium tolerance more severe (13, 22-25), opium by itself could induce brain apoptosis which may be associated with defects in some parts of brain function (26)

neuroplastic and neurotrophic changes are those cellular level changes due to repeated opium exposure that show themselves as different response to opium and opioid agents; these changes are not as much severe as apoptosis; however, they create abnormal patterns of brain function and are so called "pronociceptive changes" (27, 28).

Spinal cord related mechanisms: these are including ascending and descending pathways in the spinal cord and also, local spinal neuronal circuits. Generally speaking, we could classify them into the following mechanisms: pain elicited through descending facilitation which is a main source of spinal cord-elicited pain in opium abusers (17, 29), up-regulation of spinal dynorphin which could also have interactions with bradykinin receptors; the final result would be aggravated hyperalgesia with neuroexcitatory effect and the resulting pronociceptive pain in the spinal cord (29, 30), excitatory neurotransmitters which their release induces severe pain through spinal cord mechanisms (29, 30), role of mitogen activated protein kinase (MAP kinase) family, especially the role of TGF-β activated kinase 1 in inducing pain through spinal cord (16, 30).

peripheral nervous system related mechanisms: the peripheral nerves are subject to important changes in opium abusers that mandate more sophisticated attention; the current trend of research is focused on "silencing" the peripheral nerves to improve quality of anesthesia; though the majority of these findings are still in the research phase and have not entered the clinical era (31, 32); these research studies mainly involve: transient receptor potential (TRP) channel family especially TRPV1 (transient receptor potential vanilloid 1) antagonists and TRPA1 transient receptor potential ankyrin (TRPA1) antagonists; it is now demonstrated that pain signaling in peripheral nerves is mainly done through molecular detectors or transducers of TRP family (especially TRPV1 and TRPA1) and this is why TRPV1 antagonists and TRPA1 antagonists have a great role in pain control; in opium abusers we will use possibly in the next years such these drugs to overcome their pain management challenge; some drugs like N-ethyl lidocaine (QX-314) or capsaicin-derived pharmacologic agents work through these channels (32-34), Toll-like receptor (TLR) 5 stimulation which is among the very novel therapies for pain management in some chronic pain patients like opium abusers; which treats pain by targeting TLR-5 in peripheral nerve endings. Molecules like odanacatib (ODN; a cathepsin K inhibitor), flagellin or QX-314 act through these mechanisms (20, 34-36).

Preoperative period: before the operation

The opium abuser patients often have a number of comorbidities, including increased risk for cardiovascular disease especially coronary artery disease and other cardiac problems (6, 37-40), respiratory problems especially periods of hypoxia and lung cancers, obstructive and restrictive diseases of the lungs (6, 41-44), nutritional and gastrointestinal comorbidities including a wide range of lesions starting from erosions and lesions in the upper GI tract to peptic ulcers and increased occurrence of GI cancers (45-48); also, other system diseases (all related to abuse of opium) are more frequently seen in preoperative evaluation of these patients. In general,

opium abusers are more critical compared with general population regarding health issues (49). These studies have demonstrated that patients with history of opium abuse are at increased risk for underlying diseases which is a challenging issue for anesthesiologists who have to manage the patient and to prepare the patient for the operation (3, 6, 37).

Intraoperative period: at the time of the surgical procedure

During the operation, opium abusers need increased anesthetic drugs to tolerate surgery; in fact, the studies related to these patients have demonstrated some clinical findings that confirm the findings in animal studies or other basic researches: chronic opium abuse is a major etiology for receptor changes regarding sensation and pain perception through different mechanisms explained in previous paragraphs. These changes make the opium abusers more resistant to both opioid analgesics and non-opioid analgesics (like local anesthetics); a clinical finding in concordance with other studies demonstrating the effects of chronic opium abuse on the cellular mechanisms of pain sensation and the bizarre, wide changes in the pain perception structures of opium abusers (50-58).

The problem with these patients during the intraoperative period is that excessive opioid use results in increased chance for postoperative apnea and also, delayed emergence from anesthesia after termination of surgery (59). However, a number of other anesthetic drugs have been used successfully in opium abusers with good results; including ketamine, dexmedetomidine and clonidine in order to replace the commonly used analgesic agents, i.e. opioid derivative (51, 60-63):

- Dexmedetomidine: among the above, dexmedetomidine could be really promising with both opium sparing effects and CNS protecting mechanisms. Dexmedetomidine decreases the needed analgesic requirements in the perioperative period and also, has the property to manage opium abusers during perioperative period; especially the opioid induced hyperalgesia phenomenon; studies have demonstrated that dexmedetomidine could be used for treatment of opium withdrawal syndrome (64-68).
- Clonidine has similar chemical properties with dexmedetomidine while it is not exactly the same; however, clonidine and lofexidine, both alpha 2 agonists, could be used for treatment of opium withdrawal in opium abusers (67, 69).
- Ketamine could be used in opium abusers with fewer respiratory depression events leading to opioid sparing results; however, pain control with ketamine may be associated with a number of delirious states that should elicit cautious when using this agent; the clinical solution is to use ketamine as low dose and infusion in order to prevent the untoward effects as much as possible and to have appropriate analgesic properties (51, 60, 70).
- Paracetamol has an efficacious profile for these patients acting through non-opioid analgesic mechanisms.

In opium abusers, there is an alternative approach, and this alternative approach is to use regional anesthesia; including spinal, epidural and other methods of regional anesthesia; however, the growing bulk of evidence demonstrates that opium abusers have cross-tolerance to local anesthetics, mainly including lidocaine and bupivacaine; although, the mechanism for tolerance is similar between these agents and possibly other forms of local anesthetics are similar regarding the tolerance phenomenon; this clinical and pharmacological phenomenon presents clinically as shortened duration of action (23, 50, 52, 54, 71). This cross tolerance is a very real problem, encountered both clinically and proved in basic studies; the underlying mechanism stands on the basis of the plastic neuronal changes in the spinal cord which create tolerance to both opioids and local anesthetics (50, 52, 53).

A number of adjuvant drugs have been used with relatively successful results leading to improved regional anesthesia duration and increased analgesic properties; some studies focus on adjuvant drugs to local anesthetics in order to improve their length of analgesia in opium abusers to overcome the rescue analgesia properties (53, 72-80): first of all, opioids combined with local anesthetics in regional anesthesia improve the analgesic potency and decrease the rescue analgesic requirements, the safety profiles for most of these drugs are well established; however, sometimes we need to be more cautious to consider their safety profile, other pharmaceuticals, including magnesium sulfate, neostigmine, dexmedetomidine,

paracetamol, midazolam and others agents have been used for regional anesthesia with acceptable levels of success (53, 61, 62, 81-85).

Final problem with opium is that it may be "significant allergen" and may create some forms of anaphylaxis in operating room; the mechanism of allergy is mainly anaphylaxis and other types of immune reaction are not much common; at times, impaired hemodynamics may ensue; even when opium is used as an oral agent and could cause hypotension (86, 87).

Postoperative period: after surgery

There are a few problems in these patients during the postoperative period. First of all, these patients need much more analgesic than the others; so, care should be given to tailor their analgesic needs in such a way that prevents potential respiratory problems on one hand and their history of opium abuse with the resulting opioid tolerance on the other hand. As mentioned above, other drugs are now available that could help us manage postoperative pain in opium abusers with the use of these "novel, non-addictive or less-addictive pain medications" leading to decreased use of opium abuse (88).

However, increased analgesic requirements should not lead the management team to consider any obligatory withdrawal protocol; since the postoperative period is not a suitable clinical interval for decreasing the demands of these patients for analgesia.

Nonetheless, the increased pain perception and increased analgesic requirements are not the only problems with these patients; increased risk of postoperative delirium and postoperative cognitive disorders are among the other major clinical challenges in the postoperative period for these patients (89, 90). A number of different agents have been introduced (91). Dexmedetomidine is a synthetic novel alpha 2 agonist which could help us manage these patients in order to reduce opium dose and meanwhile, to decrease the chance for postoperative cognitive dysfunction (64, 92-94). Other agents like midazolam, clonidine or chlorpromazine could also be effective when administered as postoperative on-demand patient controlled analgesia infusion (95).

Conclusion

Opium abuse is still a major clinical challenge and we need work much more in order to improve our clinical outcomes; though there are a number of major problems during the perioperative period for opium abusing patients, still some promising points exist which help us look at future with constructive and hopeful inspirations; in summary, we can mention the followings:

1- The newly developed drugs like dexmedetomidine could improve the future of anesthesia for all patients including opium abusers in order to decrease the chance of untoward complications (like respiratory depression and postoperative delirium) while creating good analgesia with acceptable level of patient satisfaction.

2- Other older drugs like clonidine, magnesium and ketamine have demonstrated relatively good results for opium abusers with opium sparing effects.

3- Regional techniques using local anesthetic agents could have more efficacy with the help of adjuvant drugs.

Translational medicine would help us very much in near future, possibly by introducing novel drugs that treat pain through non-conventional methods including cellular and sub-cellular modification of pain and also, reversing the effects of chronic opium abuse, in order to manage these patients more efficiently (21).

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

Laboratory Diagnosis of Congenital Factor V Deficiency, Routine, Specific Coagulation Tests with Molecular Methods

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Abstract

Congenital Factor V (FV) deficiency is a rare bleeding disorder that inherit in autosomal recessive manner. Diagnosis of FV deficiency (FVD) is made by routine coagulation tests, FV activity and molecular analysis. In patients with FVD, routine coagulation tests including activated partial thromboplastin time (APTT), prothrombin time (PT), and even bleeding time (BT) are prolonged while thrombin time (TT) is normal. FV activity assay can use for confirmation of diagnosis as well as for differential diagnosis with acquired forms of disease. Mixing study can be used for screening of inhibitor against FV. In this situation, addition of normal plasma cannot correct prolonged PT and PTT while in congenital FVD prolongation is corrected. Molecular diagnosis of FVD is straight forward but due to large size of FV gene and genetic variability molecular diagnosis is restricted to research laboratory.

Keywords: Factor V deficiency, Diagnosis, rare bleeding disorder

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Introduction

Blood Coagulation factor V (FV) known as a labile factor or proaccelarin first discovered by Paul Owren in 1943 through a study on a woman that affected by hemophilia like syndrome. This protein has an essential role in hemostasis as it acts as a non-enzymatic cofactor in prothrombinase complex. It has vital role in down regulation of factor VIII by increasing the effect of activated protein C .Therefore this coagulation factor has pro and anticoagulant activity 1).

Factor V deficiency

Congenital factor V (FV) deficiency is a rare hemorrhagic disorder with estimated incidence of 1 per 1 million (1). There are more than 200 patients with FVD were diagnosed up to now. Since disorder transmits in an autosomal recessive manner, distribution of disease in both sex is equal and higher frequency of disorder in any especial area was not reported but such other autosomal recessive disorders, areas with high rate of consanguineous marriage has a higher incidence of disorder (2, 3). FV has a gene with 74 kb length on long arm of chromosome 1 and more than 100 different disease-causing mutations such as nonsense, frame shift, missense, and splice-site mutations were observed throughout the gene. Moreover, approximately 900 polymorphisms were described in FV gene (1, 3). Based on residual FV activity in plasma, FVD divided into three groups of severe with undetectable FV activity, moderate with less than 10% activity and finally, mild FVD with 10% or more than 10% FV activity. No direct correlation was observed between factor activity and severity of bleeding episodes in patients with FVD (4). A wide spectrum of clinical presentations was

Table 1: Results of coagulation tests in patients with factor V deficiency.

Test	Patient	Normal Value
Bleeding Time (BT) (template) (min)	5-20	10>
Thrombin Time (TT) (sec)	10-16	10-16
Prothrombin Time (PT) (sec)	40-50	10-13
Activated Partial Thromboplastin Time (APTT) (sec)	50-60	28-35
Factor V activity (%) Mild	10 ≤	70-150
Moderate	< 10	
Severe	Undetectable level	

observed in patients with FVD. This bleeding episodes can be as mild as recurrent epistaxis or can be a life threatening bleeding diathesis such as central nervous system (CNS) bleeding but this kind of bleedings are less common in FVD and are more common in patients with other rare bleeding disorder especially factor XIII deficiency (5, 6). Diagnosis of FVD can be made based on routine coagulation tests including activated partial thromboplastin time (APTT), Prothrombin time (PT) as well as more specific assay such as FV activity and antigen assays and finally by molecular analysis and determination of FVD underline mutation. Therefore, in this study, we presented different required tests for diagnosis of FVD including routine coagulation tests, FV antigen and activity assays as well as FV inhibitor detection and assay and molecular diagnosis of FVD (7).

Diagnosis of factor V deficiency

A set of clinical presentations, family history and laboratory assessment is useful for diagnosis of FVD. Patient with continues bleeding suspected to FVD is examined by routine coagulation tests including Bleeding Time (BT), Thrombin Time (TT) Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and platelet count(1, 3). Since FV is a part of common pathway, a patient with FVD has a normal TT and platelet count but a prolonged APTT and PT. This situation should be distinguished from acquired form of disease that are result from inhibitor development against coagulation FV or acquired form due to liver diseases. In severe FVD, BT can increase (4, 7). Combined deficiency of factor V-VIII always should be in mind to distinguish from isolated FVD. With complementary tests, all of these situations can be distinguished from FVD (7). Screening test for present of inhibitor can rule out this phenomenon. Liver function tests (LFT) with appropriate additional assessments can determine patients liver situation. FVIII coagulant activity should always be measured in all suspected patients to FVD in order to exclude the combined deficiency of FV and factor VIII (FVIII). In combined FV and FVIII deficiency low levels of both factors (usually 5–20%) is observed (7, 8). Moreover, in patients with combined FV and FVIII deficiency PT and PTT is mild to moderately be increased.

Molecular analysis and determination of underline mutation in FV gene is straight forward but because a wide spectrum of mutation was observed in patients with FVD, molecular diagnosis is not applied in clinical laboratories (8, 9).

Routine coagulation tests in factor V deficiency

At the baseline, each patient with continues bleeding can be screened by routine coagulation tests. Among these routine tests, PT and PTT were more important for coagulation factor deficiency and different coagulation factor deficiency can lead to prolongation of one or both of these tests. Prolongation of each of these tests can be further followed by more specific tests to determined underlined factor deficiency (10).

Prolonged aPTT test is observed in the deficiency of factors that belong to common (X, V, II and fibrinogen) and intrinsic (XI, IX and VIII) pathways of coagulation cascade as well as during medication by heparin. PT test evaluates the coagulation factors involved in the extrinsic (factor VII) and common (factors X, V, II and fibrinogen) pathways of coagulation cascade, and is also used for monitoring of warfarin therapy both of PT and PTT are prolong in patients with FVD but simultaneous prolongation of these tests can be observed in deficiency of each factor in common pathway (factors X, V, II, I) (7, 8, 11). In this situation, addition of normal plasma to patient's plasma can correct prolonged PT and PTT. In FVD, if normal plasma that was absorbed by aluminum hydroxide or barium salts is added to patient's plasma and PT was corrected diagnosis of FVD is suspected. Another interesting finding in FVD is prolongation of BT. Precise, cause of this prolonged BT is not clear but it can attribute to platelet FV (7). Approximately 20% of plasma FV is absorbed in platelet α granules. This platelet FV is a site for activated Factor X (FXa) but the precise mechanism of this prolonged BT with platelet FV is not clear. Inhibitor development is a rare phenomenon in patients with FVD or extremely rare in healthy individuals. This inhibitor against FV led to prolongation of both PT and PTT (7, 8). In contrast to FVD that prolonged PT and PTT was corrected by addition of normal plasma, in cases with inhibitor against FV, This is not corrected in similar situation. It's worth noting, that inhibitor against FV in congenital FVD is IgG that similar to inhibitor against factor VIII is time dependent (12).

Factor V activity and antigen assays

In a suspected patient to FVD with a prolonged PT and PTT next step is performing a FV activity assay. Based on factor activity, patients with FVD is divided to severe (with undetectable level of factor V), moderate (<10%) and mild (10≤) forms. Several methods including manual and automated methods were described that each of them has their own advantages and disadvantages (7, 8).

If a patient suspected to FVD with prolonged PT and PTT, next step to achieve a definitive diagnosis of the disease is to perform a FV activity (7).

FV specific PT assays using known FV deficient plasma can confirm the deficiency and determine the approximate functional factor levels. FV antigen levels (FV: Ag) can be determined by ELISA method. In type I of FVD, both FV activity and antigen are decreased but in type II FV activity is decreased but antigen, level is normal or near the normal (4, 7).

Molecular diagnosis of factor V deficiency

FV protein is encoded by large gene with 74kb length on large arm of chromosome 1 (1q24.2). More than 100 different mutations were observed in patients with FVD. A variety of gene defects including nonsense and missense mutations, insertions, deletions, and splice-site mutations were observed in patients with FVD (13). About 50% of mutations were missense and a large number of nonsense mutations. These different mutations were scattered throughout the FV gene without any recurrent gene defect that can be used for diagnosis of FVD. Since FV gene is large and complex with 25 exons, and diversity of FV gene defects, molecular diagnosis of FVD is not entered in clinical laboratory and is restricted to research laboratory (9, 11).

Conclusion

Taking all aforementioned in to account it can be concluded that diagnosis of FV

deficiency was done by routine coagulation laboratory tests and confirmed by measurement of factor activity. In addition molecular basis can be helpful in diagnosis of disorder. Determination of underline mutation allocated to each region around world can be used as a complementary test in diagnosis and also can be vital in prenatal diagnosis and preventing of distribution of disease.

Acknowledgment

We dedicate this work to all patients with bleeding disorders.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

Dexmedetomidine Mechanism of Action: an Update

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Abstract

Dexmedetomidine is selective agonist for α2 receptors in the central nervous system and other organs. At present, it is used as a sedative and analgesic medicine after operations. Several studies have provided evidence for new mechanism of action of dexmedetomidine. Here we reviewed the current understanding about dexmedetomidine mechanism of action involved in neuroprotection and ischemia-reperfusion injuries.

Keywords: Dexmedetomidine, neuroprotection, reperfusion injury

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Introduction

Dexmedetomidine (PRECEDEX) is an imidazole derivative that is a highly selective α2 receptor agonist. Activation of the α2 adrenergic receptors by dexmedetomidine leads to both sedation and analgesia; with negligible respiratory and cardiovascular side effects (1). Fresh experiments have provided evidence about neuroprotective properties of dexmedetomidine which can attenuate delirium, preserve sleep architecture and preserve ventilatory drive (2,3). Beyond its effects in the central nervous system, recent studies have shown efficacy of dexmedetomidine against ischemia-reperfusion injury, and against injuries following organ transplantation (4, 5).

α 2 receptor

Dexmedetomidine is highly selective for α 2 receptors. Analgesic effects of dexmedetomidine are achieved through negative feedback control found at the presynaptic level of autonomic function and in some cases in sensory neurons. Dexmedetomidine diminishes α 2 activation with release of norepinephrine from these nerve endings and other co-transmitters which are important in signal transduction (6, 7). Presynaptic that respond to the primary transmitter substance released by nerve ending are called auto-receptors (1). α 2 belongs to the family of G protein-coupled receptors (GPCRs). GPCRs are coupled by G proteins to the various effector proteins including phospholipase C (PLC) and adenylyl cyclase (AC) whose activities are regulated by those receptors. G protein is a heterotrimer consisting of α, β, and γ subunits. GPCR activation causes production of guanosine triphosphate (GTP) from guanosine diphosphate (GDP). GTP binds to α subunit and causes dissociation from two subunits. The activated GTP-bound α subunit then regulates the activity of AC. α 2 receptors inhibit adenylyl cyclase activity and cause decrease of cyclic adenosine monophosphate (cAMP) levels (8, 9). α 2–mediated inhibition of adenylyl cyclase use other signaling pathways, including

regulation of ion channel activities and the activities of important enzymes involved in signal transduction. In addition to CNS, receptors for α2 are found platelets, the liver, pancreas, kidney, eye and heart. From an anesthesiologist point of view, neuronal hyperpolarization is a key element in the mechanism of action of dexmedetomidine and is achieved by efflux of potassium and suppression of calcium entry. Loss of intracellular potassium and inhibition of calcium entry suppress neuronal firing and can inhibit signal transduction (10, 11).

Dexmedetomidine and neuroprotection

There is an increasing concern regarding the risk of anesthetic-induced developmental neurotoxicity (AIDN) in children. Numerous studies in animals have shown that general anesthetic agents not only induce neuroapoptosis, but also affect other neurodevelopmental processes in the developing brain. Anesthetic exposure induces apoptosis and neurodegeneration in a dose and time-dependent fashion (12). In the developing brain, especially during synaptogenesis, the intracellular concentration of Cl^- is high. Activation of GABA A receptor results in Cl^- efflux and depolarization of the neuron. Depolarization mediates rise in intracellular calcium concentration, which reaches levels that can contribute to neuronal injury (13). Calcium overload triggers widespread apoptotic cell death in developing brain and eventually result in long-term neurobehavioral impairment (14). The mechanism of cell death triggered by anesthetic drugs involves translocation of Bax protein to the mitochondrial membranes, where it disrupts membrane permeability, allowing extra-mitochondrial leakage of cytochrome c, followed by a sequence of changes culminating in activation of caspase-3 (13,14). Dexmedetomidine neuroprotection appears to involve a decrease in caspase 3 levels, and reversal of isoflurane-induced decrease in anti-apoptotic Bcl-1, pERK1, and pERK2 protein expression in vivo (15). Neuro-inflammatory mediators such as cytokines may be involved in a number of key steps in the pathological cascade of events leading to anesthetic-induced neuronal injury. Anesthesia can induce cytokines release in the central nervous system, leading to deleterious neurodevelopmental effect. A study by Laudenbach showed that dexmedetomidine exhibited dose-dependent protection against brain matter loss in vivo and improved the neurologic functional deficit induced by the hypoxic-ischemic insult by α2 activation (16). Another study by Tung revealed dexmedetomidine attenuates neuronal injury induced by maternal propofol anesthesia in the fetal brains, providing neurocognitive protection in the offspring rats (17). Anesthetic agents (e.g., isoflurane, propofol) may cause neurodegeneration in the developing brains and impair animals' learning ability. In that study, administration of DEX significantly inhibited propofol-induced caspase-3 activation and microglial response in the fetal brains showing anti-apoptotic effects of dexmedetomidine. On the other hand, the recent studies considering the effects of anesthetic drugs on processed electroencephalogram show that dexmedetomidine has the most similar pattern with normal sleep (18,19). These studies suggest that based on more sophisticated a clinical study considering the EEG patterns, dexmedetomidine has much more favorable than other anesthetic agents. Add to this point, the neuroprotective effects of dexmedetomidine which is associated with the least amount of neuroapoptosis in developing brain; which is discussed in other parts of the manuscript.

Dexmedetomidine and ischemia-reperfusion (I/R) injury

During reperfusion several important substances are released. Heat shock proteins (HSP) can propagate inflammatory responses possible through toll-like receptor 4 (TLR4). Oxidants activate a signal transduction cascade that may engage the cell-death pathway and provoke apoptosis. These factors contribute to development of reperfusion injury (20). Continued ischemia causes cellular accumulation of Ca^{2+} and generation of oxygen free radicals. Free radicals can directly damage mitochondria and subsequently lead to interruption in ATP synthesis and cell death (21). Kip's work

showed that dexmedetomidine caused levels of catalase (CAT) and glutathione-S-transferase anti-oxidant enzymes, and malondialdehyde (MDA) to decrease and reduced I/R injury of lungs in rat (22). In addition, Yushitimioi's study demonstrated that dexmedetomidine reduced the incidence of reperfusion-induced ventricular arrhythmias in pigs (23). The inhibitory effect of DEX on the production of tumor necrosis factor-α (TNF-α) and interleukin IL-6 following endotoxin injection is noteworthy (24). DEX induces apoptosis of neutrophils and inhibits superoxide production by neutrophils in a dose dependent manner (25). A fresh experiment by Yao showed that pre-treatment with dexmedetomidine reduced kidney pathological injury, TLR4 expression, and cytokine production following orthotopic autologous liver transplantation (OALT) in rats (26).

Conclusion

Dexmedetomidine is able to reduce neuroapoptosis and neurodegeneration by its unique mechanism of action which varies extensively from its known sedative and analgesic effects. In addition, it has beneficial effects against I/R injuries

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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