Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Functionalization of MWNT-COOH by one-step reaction with (3-oxoindolin-2-ylidene) urea and in vitro antitumor study on gastric cancer

Saeed Heidari-Keshel[1], Mahdieh Entezari[2], Mostafa Rezaei-Tavirani[1], Maryam Ebrahimi[1], Majid Rezaei-Tavirani[3]

[1] Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran Iran

[2]Department of Chemistry, Islamic Azad University, Shahre-Qods Branch, Shahre-Qods, Iran

[3]Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

ABSTRACT

Aim: In this study, we tried to prepare a nano compound with a new way in functionalization as an anti-gastric cancer candidate.

Background: Functionalization of nano-tubes is a useful route for modification of their biologic properties. (3-oxoindolin-2-ylidene) urea is a chemical compound that is made of isatin, which urea can be useful in cancer studies.

Patients and methods: This compound with one-step reaction functionalized MWNT-COOH, which is a new class in modification. This product has been investigated by FT-IR, Raman and SEM. Anti-cancer investigation with human gastric cells and MTT assay test for measurement of viable cell numbers were also performed.

Results: The two bands around 2800–2900 cm^{-1}, which are seen in functionalized products are attributed to the CH stretching of MWNT–COOH defects.

Conclusion: Cellular results demonstrated that the functionalized nano-tube is a more toxic agent compared to other samples for cancer cells and can be used as a candidate material for chemotherapy.

Keywords: Functionalization, Anti-cancer, MTT, One-pot reaction.

(Please cite as: **Heidari-Keshel S, Entezari M, Rezaei-Tavirani M, Ebrahimi M, Rezaei-Tavirani M. Functionalization of MWNT-COOH by one-step reaction with (3-oxoindolin-2-ylidene) urea and in vitro antitumor study on gastric cancer. Gastroenterol Hepatol Bed Bench 2013;6(Suppl 1):S39-S44).**

Introduction

Triple Urea and Isatin derivatives show a broad spectrum of biological activities such as antibacterial, antifungal, antiviral and anticancer drug candidate (1, 2). In patients with cancer, MWNTs have potential roles in delivering pharmacologic agents, as diagnostic imaging agents to detect or treat cancerous cells. There has been significant recent progress in the development and implementation of various covalent and non-covalent functionalization methods for chemical modification and functionalization of carbon nano-tubes (3,4). Gastric cancer is one of the major health problems in Iran. The north of Iran is a high-risk area for this type of cancer (5). In this research, we tried to prepare a nano compound with a new way in functionalization as an anti-gastric cancer candidate.

Received: 29 June 2013 *Accepted*: 1 September 2013
Reprint or Correspondence: Mahdieh Entezari, PhD. Department of Chemistry, Islamic Azad University, Shahre-Qods Branch, Shahre-Qods, Iran
E-mail: mahdiehentezari63@yahoo.com

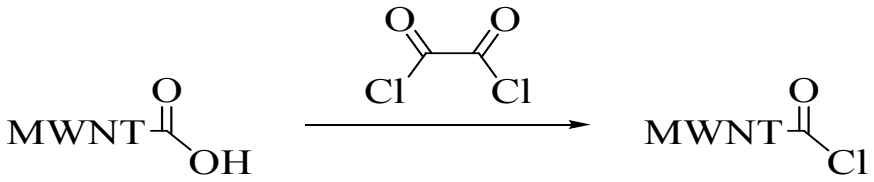

Figure 1. preparation route of MWNT–COCl

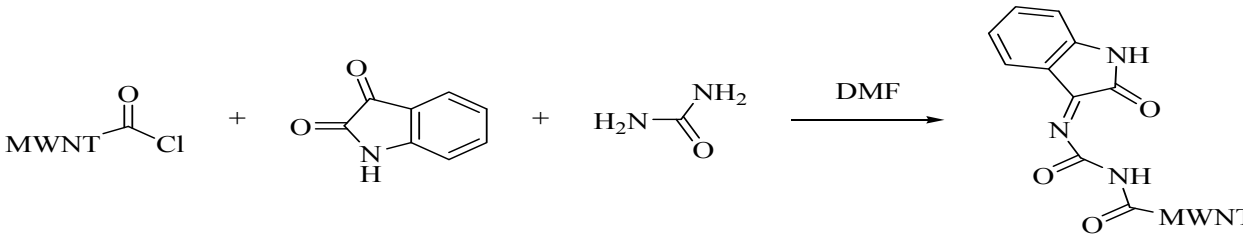

Figure 2. Preparation of Anti cancer drug Candidate by One-Step reaction of MWNT-COOH

Patients and Methods

Case presentation and management

All of the chemical materials used in this work were purchased from Merck and Fluka and was used without further purification. MWNT–COOH (95% purity, 20–30 nm; Netrino Co. Ltd) were purchased and used as received.

FT-IR

The FT-IR spectrum was recorded using KBr tablets on a Nexus 870 FT-IR spectrometer (Thermo Nicolet, Madison, WI). FT-Raman spectra were recorded on 960 ES spectrometer (Thermo Nicolet), SEM was used to study the morphology of the MWNTs. SEM measurement was carried out on the XL30 electron microscope (Philips, Amsterdam, Netherlands).

15 mg of MWNT–COOH (A) was sonicated in 30 mL of N, N-dimethylformamide (DMF) for 45 minutes to give a homogeneous suspension. Oxalyl chloride (2 mL) was added drop wise to the suspension at 0°C under nitrogen. The mixture was stirred at 0°C for two hours and followed at room temperature. Finally, the temperature was increased to 75°C and the mixture was stirred overnight to remove excess oxalyl chloride (Figure 1).

10 mg of MWNT-COCl was sonicated in 50 mL of DMF for 45 minutes to give a homogeneous suspension. The temperature was increased to 85°C and 1 mmol of isatin and urea was added to the MWNTCOCl suspension and the mixture stirred at 90°C for 10 hours. After cooling to room temperature, the mixture was filtered and washed thoroughly with THF. Subsequently, the black solid was vacuum-dried at room temperature for six hours (Figure 2).

Raman spectroscopy

Raman spectroscopy is a useful technique used to characterize structural changes of carbon nanotubes, specifically changes owing to significant sidewall modification.

Cell lines and cell culture

The human colon carcinoma cell lines (MKN-45) and human fibroblast cell lines studied in this research were obtained from National Cell Bank of Iran (NCBI) (Pasteur Institute, Tehran, Iran).

Cells were cultured in the RPMI-1640 medium supplemented with FBS (10%, v/v), streptomycin (100 µg/ml), and penicillin (100 U/ml). Cultures were maintained at 37°C in 5% CO2 and 95% air. Cultures were examined regularly. Approximately 5000 cells were seeded into 96-well cell culture plates containing 200 µl medium and incubated at 37 °C under 5% CO2 for 24 h for MTT assay and microscopy purposes. Then, the various values of essential oils (0, 1, 2, 3, 4, 5 and10 0µl) were induced in triplicate to cells for 48h. Cell survival was assessed by Colorimetric MTT assay.

Cytotoxicity analysis by MTT assay

Measuring viability and growth of cells is done with different methods. An accurate method to evaluate the survival of cells normally is the yellow tetrazolium salt assay (MTT assay). This assay is based on the cleavage of the yellow tetrazolium salt and MTT, to form a soluble blue formazan product by mitochondrial enzymes, and the amount of formazan produced is directly proportional to the number of living cells (9). In brief, this color can be measured by spectroscopic methods. There is a linear relation between the number of viable cells and absorption for each cell. This relationship allows providing accurate determination of any changes in the rate of cell proliferation. In this study, after MKN-45 and fibroblast cells were incubated for 48 hour with different values of essential oil, 20 µl MTT solution 0.5 mg/ml was added into the wells and incubation continued at 37°C for 3 to 5 hours. After this time the supernatant cells were removed, and instead of that 200 µl of DMSO solution was added for 15 min at room temperature. Finally, the percentage of cell viability was determined by ELISA reader in 570 nm. The percentage of cell viability was calculated using the equation: (mean optical density (OD) of treated cells/mean OD of control cells) × 100. Percent of cytotoxicity = 100 − percent of viability.

Results

Figure 3 shows the FTIR spectra of MWNTCOOH and functionalized MWNT. In this figure IR spectra were compared. A is IR spectrum of MWNTCOOH, the stretching frequency peaks of OH, C=O and C-O are showed at 3733, 1704 and 1204 cm^{-1} respectively. B is IR spectrum of functionalized nano-tube; peaks at 3385 and 3357 cm^{-1} are due to the N–H stretching modes. The two bands around 2800–2900 cm^{-1}, which are seen in functionalized product, are attributed to the CH stretching of MWNT–COOH defects. Carbonyl peak in the spectrum B shift to 1585 cm^{-1} (as compared with 1704 cm^{-1} in spectrum A) is a result of amid linkage formation. The bands around 1375-1277 cm^{-1} correspond to the C-N stretching mode in amides. The band around 1690 cm^{-1},which is seen in functionalized product is attributed to the C=N (6-8). Raman spectroscopy is a useful technique used to characterize structural changes of carbon nanotubes, specifically changes owing to significant sidewall modification. As can be seen in Figure 4, the characteristic peaks of MWNT tangential modes. We observed an increase in the ratio of intensities R=ID/IG in products, which is a reason for functionalization of MWNT-COOH (9-12).

Scanning electron microscopy (SEM) technique was used to look at the morphology of functionalized nano-tubes, which is shown in Figure 5 (13). SEM results showed that the MWNT–COOH (A) has a smooth surface and the products have rough surface. The in vitro cytotoxicities of MWNT-COOH as well as the corresponding functionalized MWNTs against tumor cell lines human gastric cancer (MKN45) were determined by using the MTT microculture colorimetric assay.

MKN-45 cells were seeded (15000 cells per well) into 96-wells flat-bottom microtiter plates

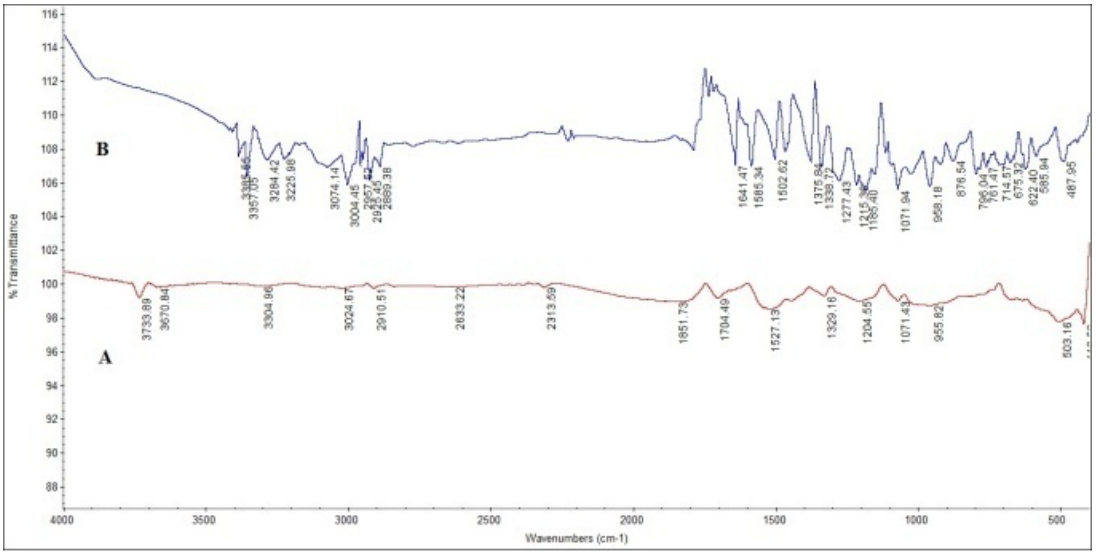

Figure 3. FT-IR spectra of MWNT–COOH (A) and functionalized product (B)

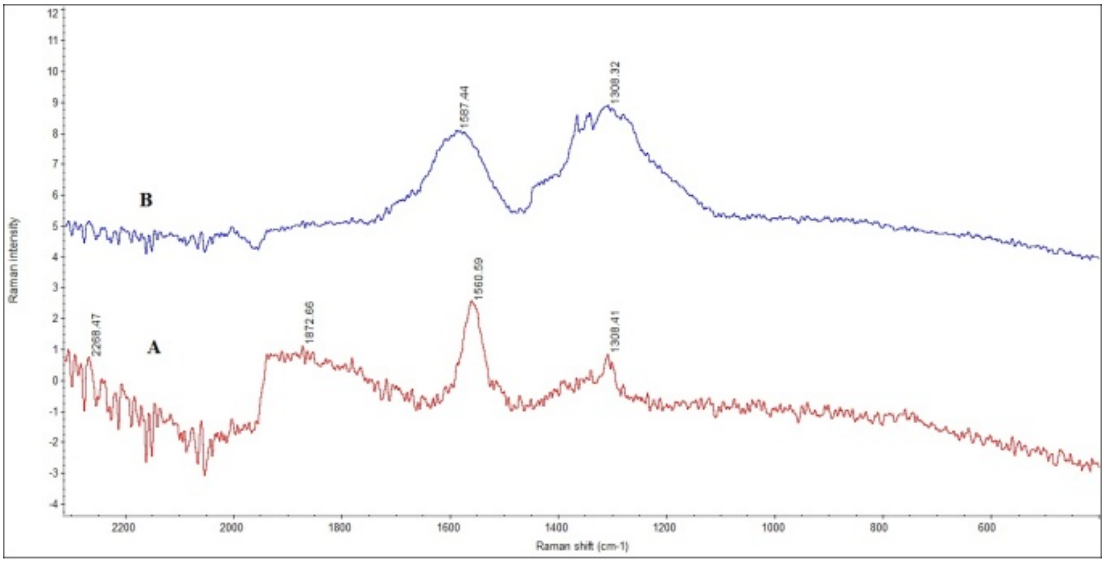

Figure 4. Raman spectra of MWNT–COOH (A) and functionalized product (B)

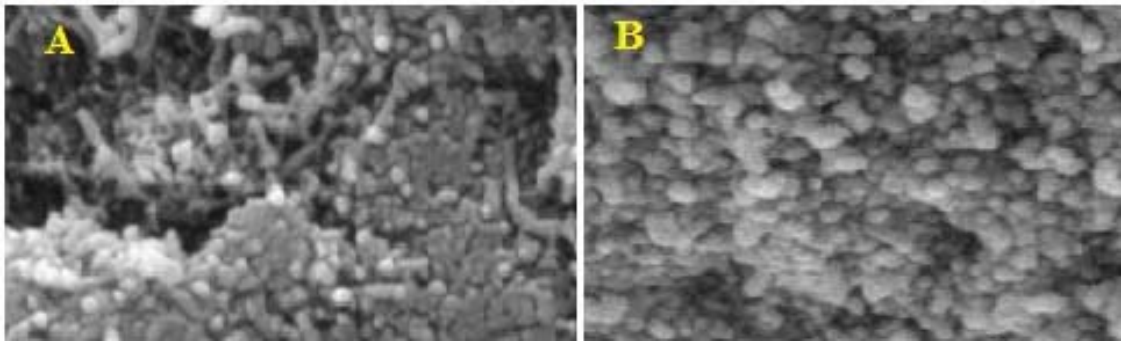

Figure 5. SEM spectra of MWNT–COOH (A) and functionalized product (B)

and incubated for 4h prior to the addition of filtered 3 different concentrations of the studied compounds. Final concentrations achieved in treated wells were 0.001, 0.01 and 0.1μg/mL. The

optical density (OD) value was defined as the absorbance of each individual as well. Finally, the absorbance of 570 nm (test wavelength) and with a reference filter of 630 nm was measured. All experiments were performed three times and the viability was calculated and showed in figure 6 (14-15).

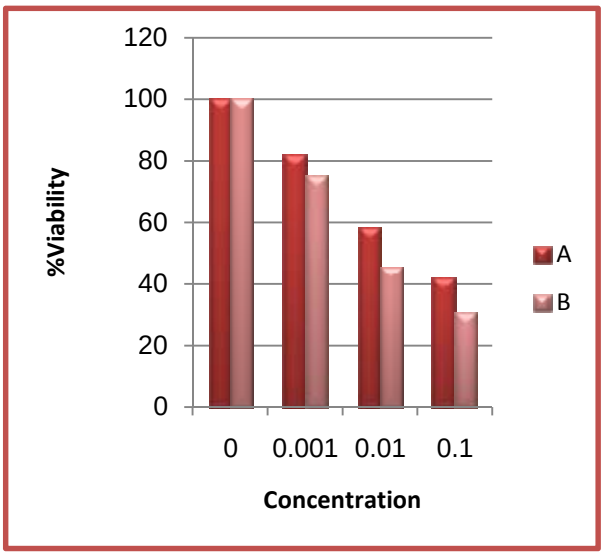

Figure 6. Cytotoxicity graphs from typical MTT assay showing the effect of MWNT-COOH A) and functionalized MWNT (B) on the viability of MKN-45

Discussion

CNTs have been attracting much attention from chemists and scientists owing to their electronic, mechanical, optical and chemical characteristics. Multiwalled CNTs (MWNTs) are more attractive than single walled CNTs because of their relatively low production costs and availability in large quantities. In recent years, the chemical functionalization of CNTs has been more interesting, because it allows the modification of nano-tube surface for subsequent alignment. These surface modifications play an important role for application of nano-tubes in composite, sensors and many other fields. The chemical modifications of CNTs have been well summarized in several review articles. Amines are among the reagents that have drawn the greatest attention. We have designed and synthesized novel derivatives of (3-oxoindolin-2-ylidene) urea that have a potential use for cellular toxicity especially cancerous cells. The route for preparation is a one-pot three component that is a new way for modification of MWNTs. The reported results demonstrate the functionalization of MWCNT. Cellular results demonstrated that the functionalized nano-tube is a more toxic agent compared to other samples for cancer cells and can be used as a candidate material for chemotherapy.

Cancer is a frightening disease for the world. In this project we tried to make compounds against gastric cancer. Hopefully, others will benefit from this article and one day, cancer will be eradicated worldwide.

Acknowledgements

The financial and encouragement support provided by Research Vice Presidency of Shahre-Qods Branch, Islamic Azad University.

References

1. Madhavaa G, Venkata Subbaiahb K, Sreenivasulu R. Synthesis of novel urea and thiourea derivatives of diphenylphosphoramidate and their antimicrobial activity, Scholars Research Library. Der Pharmacia Lettre 2012; 4:1194-201.

2. Chen GX, Kim HS, Park BH, Yoon JS. Controlled functionalization of multiwalled carbon nanotubes with various molecular-weight poly(L-lactic acid). J Phys Chem B 2005;109:22237-43.

3. Banerjee, S, Hemraj-Benny, T, Wong, S, Covalent Surface Chemistry of Single-Walled Carbon Nanotubes. Adv Mater 2005;17: 17-29.

4. Tasis D, Tagmatarchis N, Bianco A, Prato M. Chemistry of carbon nanotubes. Chem Rev 2006;106: 1105-36.

5. Malekzadeh R, Derakhshan MH, Malekzadeh Z. Gastric Cancer in Iran: Epidemiology and Risk Factor. Arch Iranian Med 2009; 12: 576 – 83.

6. Hsu-Chiang K, Chen-Chi MM, Wei-Ping Ch, Siu-Ming Y, Hsin-Ho W, Tzong-Ming L. Synthesis,

thermal, me-chanical and rheological properties of multiwall carbon nanotube/waterborne polyurethane nanocomposite. Compos Sci Technol 2005;65:1703-10.

7. Stevens JL, Huang AY, Peng H, Chiang IW, Khabashesku VN, Margrave JL. Sidewall Amino-Functionalization of Single-Walled Carbon Nanotubes through Fluorination and Subsequent Reactions with Terminal Diamines. Nano Lettb 2003; 3:331-36

8. Peng H, Alemany LB, Margrave JL, Khabashesku VN. Sidewall carboxylic acid functionalization of single-walled carbon nanotubes. J Am Chem Soc 2003;125: 15174-82.

9. Dettlaff-Weglikowska U, Benoit JM, Chiu PW, Graupner R, Lebedkin S, Roth S. Chemical Functionalization of Single Walled Carbon Nanotubes, Appl Phys 2002;2:497-501

10. Hamon MA, Hu H, Bhowmik P. End-group and defect analysis of soluble single-walled carbon nanotubes. Chem Phys Lett 2001;347-48.

11. Jorio A, Saito R, Dresselhaus G, Dresselhaus MS. Determination of nanotubes properties by Raman spectroscopy. Philos Trans A Math Phys Eng Sc 2004;362: 2311-36.

12. Rao AM, Richter E, Bandow S, Chase B, Eklund PC, Williams KA, Fang S, Dresselhaus G, Dresselhaus MS. Diameter-Selective Raman Scattering from Vibrational Modes in Carbon Nanotubes. Science 1997; 275: 187-91.

13. Al Faraj A, Fauvelle F, Luciani N, Lacroix G, Levy M, Crémillieux Y, et al. In vivo biodistribution and biological impact of injected carbon nanotubes using magnetic resonance techniques. Int J Nanomedicine 2011;6:351-61.

14. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods1983**;** 65: 55-63

15. Heo DS, Park JG, Hata K. Evaluation of Tetrazolium–Based Semiautomatic Colorimetric assay for measurment of human antitumor cytotoxicity. Cancer Res 1990; 50: 3681-90.