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Original Article

FTIR Determination of Miconazole Effects on Mice Fetus Brain Tissue

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Abstract

Miconazole is an imidazole antifungal agent, commonly applied topically to the skin or mucous membranes. The aim of this study was to examine the alternative method for gaining mechanism or the bimolecular changes caused by the possible teratogenic effects of Miconazole on mice fetus brain tissue using FTIR-Microspectroscopy. The mice were injected with Miconazole (60 mg/Kg) on gestation day 9. Fetuses were dissected on day 15 of gestation and morphological and histological studies on the fetus were carried out. Sections (10 μm) of control and Miconazole treated fetus brain tissue were used for FTIR measurement in the mid- infrared region. The results were shown by spectra 2nd derivative and also subtracting from control spectra. A lower intensity in the lipid (2800–3000 cm^{-1}) and amid I (1600–1800 cm^{-1}) regions of Miconazole treated mice fetus brain tissue was observed compared to the control mice fetus brain tissue. No major spectral shifting was observed at amide I band, amide II band and nucleic acid regions. As a conclusion, FTIR-Microspectroscopy can be a useful tool for teratogenic measurement with a unique ability to identify the modified bimolecular structures in mice fetus tissues.

Keywords: Biospectroscopy, FTIR-Microspectroscopy, Mice fetus, Miconazole, Teratogenic.

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1. Introduction

Miconazole is imidazole antifungal agent, commonly applied topically to the skin or mucous membranes. Miconazole is occasionally used intravenously to treat severe systemic mycotic infections, while local preparations are used for topical treatment of vulvovaginal candidiasis or superficial skin infections caused

by the dermatophytes and candida species [1]. The effect of Miconazole in dogs against several species of yeast has been demonstrated [2]. Coadministeration of Miconazole, ketoconazole, and fluconazole with acetazolamide to pregnant mice, all increased the frequency of forelimb ectrodactyly [3]. Metronidazole and Miconazol use for against fungi and topical treatment of common Volvo vaginal infections such as candidiasis, bacterial vaginitis and trichomonas vaginitis. Concomitant of Metronidazole and Miconazole for vaginal treatment at during pregnancy especially at organogenesis period caused polydactylyl and syndactylyl in the upper organs, skeletal defects, cleft palate, the rib deformity, cranial deformity, dilatation of the renal pelvis and bladder, etc [4-11].

The researchers (both clinical and non-clinical) in biological studies have been attracted to detect malignancy and cancer by applications of spectroscopic techniques [12]. The identification and chemical distribution of cellular materials and tissue components and also essential information to understand biological activity are enabled by biochemical IR micro spectroscopic imaging [13-18]. We have started a series of investigations to evaluate the applicability of FTIR spectroscopy for the determination of different agents teratogenicity such as Phenobarbital and Metronidazole effects on mice fetus liver and brain tissues [19-20]. The aim of this study was to examine the alternative method for gaining mechanism or the bimolecular changes caused by the possible teratogenic effects of Miconazole on mice fetus brain tissue using FTIR microspectroscopy.

1. Materials and Methods

2.1. Sample Preparation for FTIR Microspectroscopy

Miconazole from Sigma co. was purchased. Adult mouse (10-12 weeks) weighting 20g were obtained from Razi institute, Iran. The mouse was fed with a standard diet with water and libitum, and kept in a room with controlled light (12:12, dark: light), temperature (22±10 C), relative humidity (40-50%) and ventilation (15 air changes per hour). They were allowed to adapt to their environment for 1 week prior to the experiments. The mouse was randomly mated and for emphasis of pregnancy were assessed vaginal plaque after their mating. Then, pregnant mouse was divided into two groups and treated as follows: The control group (3 mice) received anything and the test group (3 mice) received Miconazole (60 mg/kg) intraperitoneally on gestation day 9. Pregnant mouse was sacrificed and dissected on day 15th of gestation and morphological and histological studies on the fetus were carried out [21]. After tissue fixation with bouin fixative solution, fetus sections at a pre-defined thickness of 10 µm was performed. Slices were either thaw-mounted on a 1mm thick KBr window for IR microscopy and were mounted on conventional glass slides for haematoxylin (H) and eosin (E) staining for studying of abnormality in fetus by light microscopy [22].

2.2. FTIR Microspectroscopy (FTIR-MSP)

FTIR measurements were performed in the absorbance mode. WQF-510 Fourier transform spectrometer (Rayleigh Optics, China) equipped with a KBr beam splitter equipped with a DLaTGS (Deuterated Lantanide Triglycine Sulphate) detector and μMAX IR microscope (PIKE Technologies, USA). The spectra were scanned in the mid-IR range from 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}. 100 scans were coded for each spectrum and the spectra were ratio against the background spectrum.

2.3. Data Processing and Statistical Analysis

The data were analyzed using Main FTOS IR software (routine software on the FTIR equipment). The spectra were recorded from several sites on the sections of mouse fetus brain tissue and an average spectrum from the all spectra was computed. All spectra were baseline corrected and normalized to the band at 1657 cm^{-1} (amide I peak). Second order derivatives were also calculated to show more details of spectral changes in specific region (amid I band; 1600-1700 cm^{-1}).

3. Results and Discussion

FTIR- MSP spectrum of Control and Miconazole treated mouse fetus brain tissues showed in Figure 1-A. The information contained in such an IR absorption spectrum originates from all different types of biomolecules in the brain tissue, such as lipids, proteins and nucleic acids. The Miconazole treated brain tissues had a lower intensity in the lipid (2800–3000 cm^{-1} and 1463 cm^{-1}) and amid I (1600–1700 cm^{-1}) regions compared to the control brain tissue. No major spectral shifting were observed for protein amide I band at 1655 cm^{-1} (mainly C=O stretching of proteins), amide II band at 1544 cm^{-1} (C-N stretching coupled with N-H bending modes of proteins) and nucleic acids region (asymmetric PO_2^- stretching of RNA and DNA in 1220 to 1240 cm^{-1} and Symmetric phosphate PO_2^- stretching at 1077 cm^{-1}) [12].

The difference spectra; Miconazole treated tissue spectra minus control spectra [(Miconazole treated) – (Control)] was obtained; in order to monitor the intensity variations of vibrations and the result are shown in Figure 1-B. Negative features were located in the difference spectra at 1624 cm^{-1} in amide I band region and lipid regions at 1488 cm^{-1} and 2860 cm^{-1}, are related to a minor decreasing in the intensity of the mentioned bands in Miconazole treated group compared to the control group.

Second derivative resolution enhancement in the 1600-1800 cm^{-1} region were used to show more detail for amid I band (Figure 2). The Amid I band is highly sensitive to conformational changes in the secondary structure of proteins [23]. No spectral shifting were observed in secondary derivative spectra of amid I band.

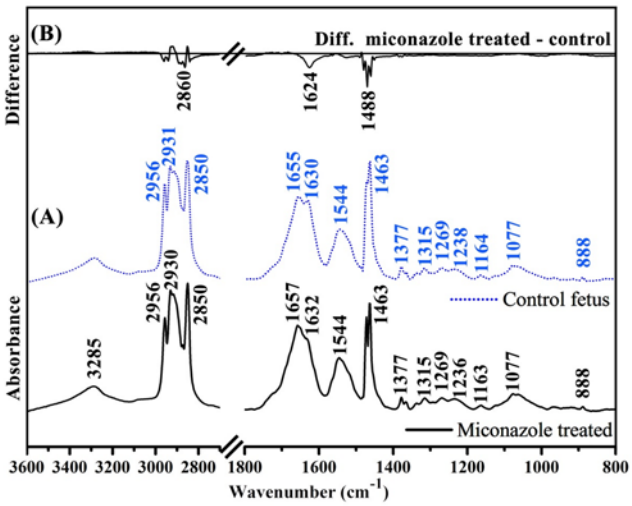

Figure 1. Mid-infrared spectra of Control and treated mouse fetus brain tissue (A), the spectra were baseline-corrected, and normalized to1655 cm^{-1} (amide I peak) band intensity. The difference spectrum was obtained by subtracting of treated spectra from the control spectra (B).

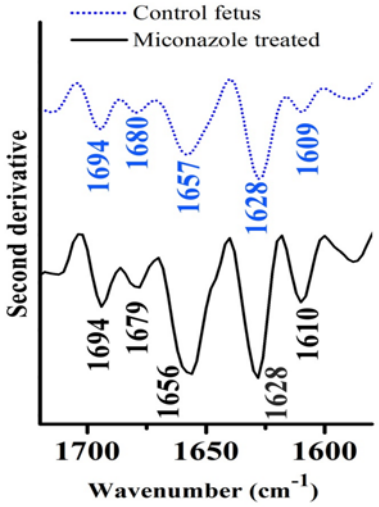

Figure 2. The 2nd derivative spectra in the 1600-1800 cm^{-1} region for Control and Miconazole treated groups.

4. Conclusion

Several foreign agents can alter normal embryonic development leading to teratogenesis. The azole agents (triazole or imidazole derivatives) are under the focus of investigation for their inducing birth defects [24]. Azole fungicides are widely used in agriculture and in human mycosis. There are teratogenic potential to induce facial, axial skeleton, and limb defects by several azole fungicides tested in laboratory animals; it was observed an increase of axial skeletal defects When Miconazole was administered to pregnant mice on days 8, 9 or 10 of gestation and also vulvovaginitis [25]. Azoles such as Miconazole are teratogenic in animal models and are under investigation for potential human developmental toxic effects [3-11 & 24-25]. For some decades, Biospectroscopy is the

main technique used to detect the exact molecular alterations of these defects. Previously, the result of investigations at the chemical bands of mice fetal tissue biomolecules and their alterations after exposure to Metronidazole and Phenobarbital drugs was expressed by using the FTIR-MSP [19-20]. The most important changes of Phenobarbital treated mice fetus brain tissue were on the β structure of proteins due to the amide I bands at 1636 cm, while extensive effects on the DNA structure were obvious for the Phenobarbital treated mice fetus liver tissues [19] and also the most variations in Metronidazole treated mice fetuses brain and liver tissues were in amid bands, nucleic acid and carbohydrate related bands [20].As shown in the result section, Miconazole induces a bit changes in the mouse fetus brain tissue. The Miconazole treated brain tissues had a lower intensity in the lipid and amid I regions compared to the control brain tissue. Since Amide I band is highly sensitive to the conformational changes in the secondary structure of proteins that's why 2^{nd} derivative was applied for further survey at this band but there wasn't any shift. No major spectral shifting was observed for protein amide I, amide II, nucleic acid and carbohydrate related regions. Actually more detail and statistical power is needed to prove and elaborate on these findings.

This work is showed no major infrastructural alterations were occurred in molecular level of brain tissue in mice fetus after the exposure of the pregnant mother by Miconazole. Furthermore, FTIR spectroscopy is proposed as a useful and rapid model in the preliminary screening for teratology studies.

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