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An experimental study of the systemic alteration of nitroimidazoles in the middle stage of embryonic development

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Abstract

Nitroimidazole compounds have been traditionally used in the veterinary and human medicine, but their uses are sometimes associated with some side effects. Toxicopathological effects of nitroimidazoles have always been a major concern. There is scant information available about the pathological alterations of nitroimidazole compounds in the embryo. The objective of this study was to determine the macroscopic and microscopic lesions of metronidazole in the chicken embryo. Fertile broiler eggs were divided into two equal treatment groups as follows: group 1: saline-injected group, the eggs were injected with sterile physiological saline solution into the chorioallantoic sac. Group 2, individuals were injected with metronidazole at a dosage of 25 mg/Kg egg-weight three times into the chorioallantoic sac. Macroscopically, the embryos were stunted. The feet and wings were small and the feather formation was seriously affected. In this group, the embryos were affected by schistosomus reflexus. Microscopically, all organs were hyperemic. Based on macroscopic and microscopic findings, it is concluded that metronidazole at the above-mentioned concentration is toxic to the chicken embryo. The current study also advises caution in the extended use of nitroimidazole compounds.

Keywords: Chicken, Egg, Embryo, Metronidazole, Toxicopathology

Introduction

Nitroimidazole compounds have been used to treat parasitic infections in humans for many years. These compounds have treated protozoal disorders and some infectious diseases. They affected against *Mycobacterium tuberculosis, giardiasis*, Trypanosoma brucei, Trypanosoma cruzi, Leishmania and *trichomonal vaginitis* (Mukherjee and Boshoff 2011; Carroll et al. 2013; Pasupuleti et al. 2014; Patterson and Wyllie 2014). Metronidazole is a nitroimidazole antibiotic medication used particularly for anaerobic organisms, amoebicide and antiprotozoal. It is recognized to be active against protozoa as well as facultative anaerobes (*Helicobacter pylori* and *Gardnerella vaginalis*) and anaerobic bacteria in human (Lucasti et al. 2013; Han et al. 2014; Leitsch et al. 2014). In veterinary, metronidazole has been used for many years in the therapeutic management of trichomoniasis, giardiasis, and amebiasis. It is active against obligate anaerobic bacteria. After PO or parenteral

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administration, metronidazole is active against *Bacteroides fragilis, Melaninogenicus, Fuso-bacterium spp, Clostridium perfringens* and *other Clostridium spp* (Ahrens and Martin 2013; Dowling 2013; Giguère et al. 2013). Despite the use of nitroimidazoles in veterinary and human medicine, its use is sometimes associated with adverse effects such as neurotoxicity, muscle spasms, ataxia, convulsions, bone marrow depression and discoloration of the urine (Sweetman et al. 2009; Barry et al. 2013; Manousaridis et al. 2013; Turnbull et al. 2013; Frasca et al. 2014). Each adverse effect is due to certain nitroimidazole compounds. It was also shown that some nitroimidazole compounds cross from the placenta into the fetus with undetermined short- or long-term effect (Heisterberg 1984; Burtin et al. 1995). Although increasing consumption and production of nitroimidazoles compounds are predicted in medical and veterinary medicine, there is little information about the teratogenic and toxic effects of these compounds on the embryo. Thus, the current study aimed to determine the macroscopic and microscopic lesions of metronidazole in the chicken embryo. Furthermore, this study was performed in the chicken embryo as a model to investigate toxicopathological effects of metronidazole for the human fetus since the embryogenesis in chicken is similar to human beings. It is our belief that this experiment will provide important information about the toxic effects of nitroimidazoles compounds as a basic embryo-toxicological study.

Materials and Methods

Hatching eggs

Fertile chicken eggs (Ross 308) with the average egg-weight of 49.6 ± 0.3 g were purchased from a local breeder farm whose birds were maintained under standard environmental conditions.

Experimental protocol

Eggs were incubated at 37.7ºC and 60% relative humidity. The eggs were randomly assigned to two equal treatment groups, fifteen eggs each, as follows: Group 1: saline-injected group, embryonated eggs were injected with sterile physiological saline solution of 0.3 ml/egg into the chorioallantoic sac. Group 2: eggs were treated with metronidazole (%0.05 injectable solution, Shahid Ghazi Pharmaceutical Co, Iran) at a dosage of 25 mg/Kg egg-weight/day (0.3 ml/egg) at days 9, 10 and 11 of incubation. Embryos received treatment by direct injection into the chorioallantoic sac according to the standard techniques (Hamburger 1942; Ohta and Kidd 2001; Mukherjee and Boshoff 2011). Embryos were reincubated post-treatment and allowed to develop until day 18, after which they were collected and examined for macroscopic and microscopic lesions. All experiments were conducted according to local ethical guidelines, and were approved by the Animal Ethics Committee of the Research Council of Shahid Bahonar University, Iran.

Pathological examination

At the end of the experiment (on day 18) the embryos were humanely killed by placing on ice and then the eggs were opened at the wider end (Jacobsen et al. 2012; Tavakkoli et al. 2014). After washing in normal saline solution, embryos were observed under stereomicroscope to study any gross abnormalities on the external body surface. The membranes and chorioallantoic sac were also inspected. The muscle, liver, kidney, heart and brain were dissected out and fixed in 10% neutral buffered formalin. Following routine preparation of tissues, serial sections of paraffin-embedded tissues of 5 µm thickness were cut using a microtome (Slee-Germany), then stained with hematoxylin and eosin and studied under a light microscope.

Statistical analysis

Statistical analysis was performed using SPSS version 20. The Fisher's exact test was used to determine the significant differences in lesion occurrence between experimental groups. A P-value of <0.05 was considered as statistically significant.

Results

Macroscopic findings on the external body surface

The tissues of the embryos were normal in group 1 which treated with sterile physiological saline solution into the chorioallantoic sac (Fig.1). In metronidazole-injected group (group 2) the embryos were stunted. The feet and wings were small and the feather formation was affected, so the feathers were not formed perfectly. In these groups, the embryos were affected by schistosomus reflexus (Fig.2).

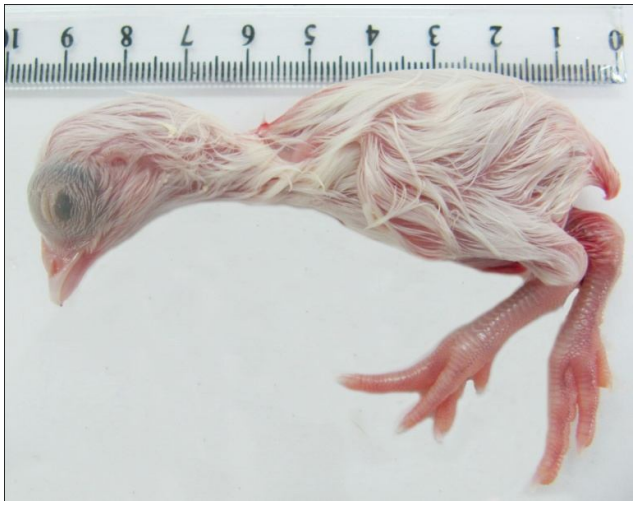

Fig. 1 The chicken embryo treated with sterile physiological saline solution into the chorioallantoic sac. The embryo is normal with no gross lesions.

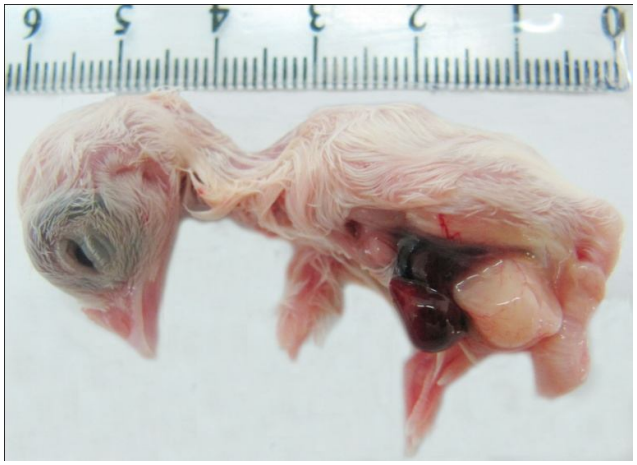

Fig. 2 The chicken embryo treated with metronidazole at a dosage of 25 mg per Kg egg-weight into the chorioallantoic sac. The embryo is stunted and affected by schistosomus reflexus. Feather abnormality, is seen on the entire body**.**

Histological findings

The embryos were normal in group 1 which treated with sterile physiological saline solution into the chorioallantoic sac. The lesions were occurred in the embryos of group 2, which received metronidazole into the chorioallantoic sac. Histopathological lesions were seen in muscle, liver, kidney, heart and brain of embryos. In these embryos, the tissues were hyperemic. Photomicrographs of the lesions are seen in figures 3 to 5.

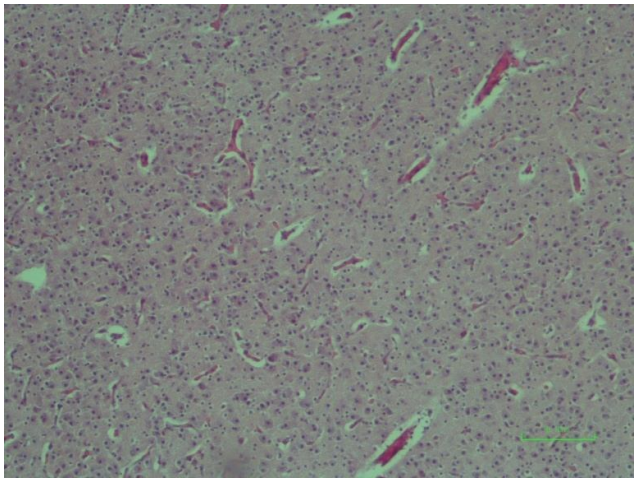

Fig. 3 Photomicrograph of the chicken embryo treated with metronidazole at a dosage of 25 mg per Kg egg-weight into the chorioallantoic sac. Hyperemic brain tissue is seen. ×100 H&E

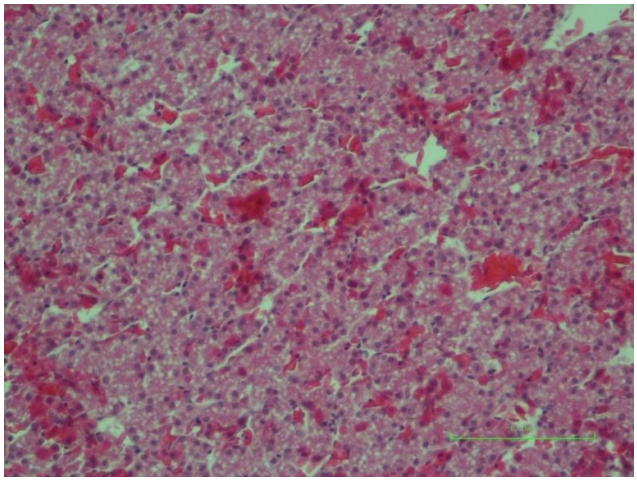

Fig. 4 Photomicrograph of the chicken embryo treated with metronidazole at a dosage of 25 mg per Kg egg-weight into the chorioallantoic sac. Hyperemic liver tissue is seen. ×200 H&E

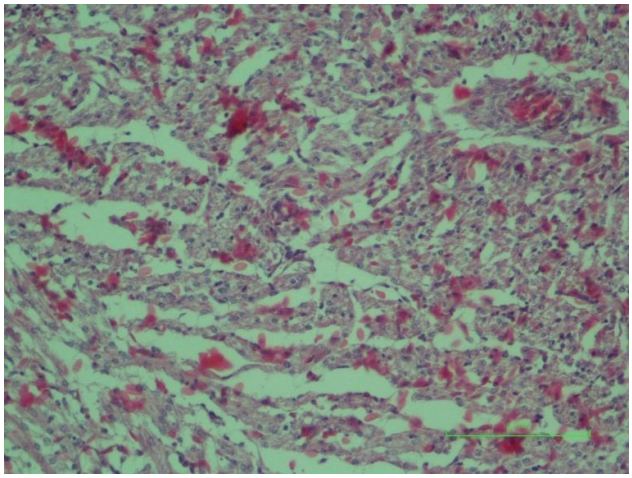

Fig. 5 Photomicrograph of the chicken embryo treated with metronidazole at a dosage of 25 mg per Kg egg-weight into the chorioallantoic sac. Hyperemic heart tissue is seen. ×200 H&E

Discussion

Determining the side effects of chemical elements in the development of chicken embryos is a useful method for studying the biological properties of chemical elements and drugs (Tavakkoli et al. 2013; Tavakkoli et al. 2014). The current study is focused on the lesions induced by administration of metronidazole in the chicken embryo. Our results clearly showed the gross and microscopic alteration in chicken embryos exposed to metronidazole. The macroscopic lesions in embryos were characterized by stunting, impaired feather formation, small feet and wings and schistosomus reflexus. Microscopic lesions were seen in the muscle, liver, kidney, heart and brain of embryos. The tissue distribution of nitroimidazoles in the avian embryo is unknown, but based on our histopathological results it was concluded that metronidazole could distribute to the muscle, liver, kidney, heart and brain of chicken embryos. In the human model, it is shown that metronidazole distributes to various tissues including, central nervous system, middle ear discharges, bile, peritoneal fluid, and fluids and tissues of the female genital tract (Bergan 1984; Lamp et al. 1999). In the brain of embryos, hyperemia was seen. Some studies suggest that metronidazole can cause neurological alteration (Bottenberg et al. 2011; Kuriyama et al. 2011; Hari et al. 2013; Sarna et al. 2013). The mechanism behind metronidazole-induced brain lesions in chicken embryo is not exactly known. Further investigations are needed to elucidate the underlying mechanisms.This study showed hepatic hyperemia in chicken embryos, which injected with metronidazole. It was reported that metronidazole may falsely elevate AST, ALT and lactate dehydrogenase (Pinches 2006). Our findings are comparable to previous reports for other nitroimidazoles compounds known to have hepato-toxic potential (Hoogenboom et al. 1991; Senousy et al. 2010; Coskun et al. 2012; Radko and Minta 2012).

In the present study, metronidazole produced histopathological alterations, mainly hyperemia of the renal tissue. Metronidazole nephrotoxicity has been reported by some authors in different species (Oda 2012). Little information is available about the renal damage induced by metronidazole in the bird embryo, and no well-described experimental model exists. It was also shown that metronidazole crosses the placenta (Heisterberg 1984; McDonald et al. 1994; Sweetman et al. 2009), so, further studies still need to be undertaken to determine the teratogenic effects of metronidazole on the development of a human fetus. In conclusion, the results of the present study describe pathological alterations induced by administration of metronidazole in chicken embryo. The current study also advises caution in the extended use of nitroimidazoles compounds. Further studies are needed to clarify the toxic effects of metronidazole on the development of vertebrate fetus.

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