doi: https://doi.org/10.52973/rcfcv-e34310 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34310

Determination of the embryotoxic effect of Metronidazole using an *in ovo* model

Determinación del efecto embriotóxico del Metronidazol mediante un modelo *in ovo*

***Muhittin Uslu*[1]*, *Rahmi Canbar*[2], *Mustafa Sedat Arslan*[3], *Harun Kızılay*[4]**

[1]*Yozgat Bozok University, Department of Laboratory and Veterinary Health, Sefaatli Vocational College. Yozgat, Türkiye.*
[2]*Necmettin Erbakan University, Faculty of Veterinary, Department of Pharmacology and Toxicology. Konya, Türkiye.*
[3]*Selcuk University, Faculty of Veterinary Medicine, Department of Anatomy. Konya, Türkiye.*
[4]*Selcuk University, Faculty of Pharmacy, Department of Pharmacology. Konya, Türkiye.*
Corresponding Author: muhittin.uslu@bozok.edu.tr

ABSTRACT

Metronidazole is an imidazole group bactericidal antibiotic used against anaerobic bacteria and some protozoa. There is no detailed information about the embryotoxic effect of Metronidazole. This study aims to determine the embryotoxic activity of Metronidazole using an *in ovo* method. A total of 210 fertile chicken eggs were placed in an incubator, divided into seven equal groups of 30. The first group was considered as the control group. On the seventh day of the study, Metronidazole was administered to the other six groups within 50 µL saline solution at doses of 250, 125, 62.5, 31.25, and 15.62 µg·egg^{-1} (5; 2.5; 1.25; 0.625; 0.312 mg·kg^{-1}). At the end of the incubation period, the eggs hatched, and the number of live and dead embryos was determined. There were no significant differences in deaths between the groups (P>0.05 in all cases). No anomaly was detected in the macroscopic morphology of the embryos. As a result, it can be stated that Metronidazole may be safe for use during pregnancy. However, it is necessary to conduct molecular and histopathological studies to investigate the effects of this drug on organogenesis, especially in mammalian embryos.

Key words: Metronidazole ; embryotoxicity; *in ovo*

RESUMEN

El metronidazol es un antibiótico bactericida del grupo de los imidazoles que se utiliza contra las bacterias anaerobias y algunos protozoos. No hay información detallada sobre el efecto embriotóxico del metronidazol. Este estudio tiene como objetivo determinar la actividad embriotóxica del metronidazol mediante un método *in ovo*. Se colocaron en una incubadora un total de 210 huevos fértiles de gallina, divididos en siete grupos iguales de 30. El primer grupo se consideró como grupo control. En el séptimo día del estudio, se administró metronidazol a los otros seis grupos dentro de 50 µL de solución salina en dosis de 250; 125; 62,5; 31,25 y 15,62 µg·huevo^{-1} (5; 2.5; 1.25; 0.625 y 0.312 mg·kg^{-1}). Al final del período de incubación, los huevos eclosionaron y se determinó el número de embriones vivos y muertos. No hubo diferencias significativas en las muertes entre los grupos (P>0.05 en todos los casos). No se detectó ninguna anomalía en la morfología macroscópica de los embriones. Como resultado, se puede afirmar que el metronidazol puede ser seguro durante el embarazo. Sin embargo, es necesario realizar estudios moleculares e histopatológicos para investigar los efectos de este fármaco sobre la organogénesis, especialmente en embriones de mamíferos.

Palabras clave: Metronidazol, embriotoxicidad, *in ovo*

Received: 19/08/2023 Accepted: 26/09/2023 Published: 01/01/2024

INTRODUCTION

Metronidazole is an imidazole group bactericidal antibiotic used to treat anaerobic and some protozoan infections (e.g., giardiasis, amoebiasis, histomonas, trichomoniasis). Although its use in animals with nutritional value is prohibited in Veterinary Medicine [1, 2, 3] it is frequently used in Human Medicine. Commercial preparation for pet clinics is licensed in Veterinary Medicine, where it is used mainly in pyometra, pyoderma, dental abscesses, and bite injuries [4].

Metronidazole causes mild to moderate gastrointestinal side effects such as diarrhea, nausea, and abdominal pain, but is well tolerated [2]. In the central nervous system, it is reported that it commonly causes dysarthria, mental changes, and ataxia, and more rarely, neurotoxicity, optic neuropathy, encephalopathy, and peripheral neuropathy [5, 6, 7]. It has been reported that the drug, which is in the IARC 2B class, has genotoxic and carcinogenic properties in animals. However, more studies are needed to reveal its genotoxic and carcinogenic properties in humans [2, 8].

Fertile chicken embryos are often preferred to mammals in investigating the embryotoxic and teratogenic effects of chemicals and drugs [9, 10, 11, 12, 13]. For this purpose, the Chick Embryotoxicity Screening Test (CHEST) test was developed [14]. Reasons for using chicken embryos in the CHEST method include the cheapness of chicken embryos, ease of application, well-known developmental stages, reproducible results, and statistical superiority over mammalian species due to the availability of large numbers of fertile chicken eggs in research. In addition, factors such as being able to carry out studies with low-tech laboratory equipment, obtaining results quickly, and similarity between the morphological development of chicken embryos and that of mammals have also been expressed as advantages [14, 15, 16]. The disadvantages are the lack of a maternal-fetal relationship typical of mammals, the possibility of false positives, and the pharmacokinetic differences caused by differences in the nature of chicken eggs. In addition to its disadvantages, species diversity is also encountered in other tests, and the closest data to the target species should be sought [15, 16].

There is no detailed information about the use of Metronidazole in either Veterinary or Human Medicine. It is defined as category B in pregnancy by the Food and Drug Administration in Human Medicine [17]. Contrarily, it is advised against using metronidazole during pregnancy due to the risk of congenital abnormalities or embryotoxicity [18]. As a result of these studies, it was hypothesized that the embryotoxic and teratogenic effects of Metronidazole would be dose dependent, using the in ovo model.

MATERIAL AND METHODS

It was obtained 210 fertile chicken eggs from a commercial enterprise (Anadolu Damizlik, Konya, Türkiye). The Selçuk University Faculty of Veterinary Experimental Animals Production And Research Center Ethics Committee approved the research procedure (2022/93). Fertile eggs were divided into seven equal groups of 30 and placed in an incubator (Imza Teknik, Konya, Türkiye). During incubation, eggs were housed under optimal maintenance conditions (37.8°C, 65% humidity, and a rotation time of 2 hours). Fertility was checked under light on the seventh day of incubation and non-fertile eggs were removed from the groups. Fertile eggs were substituted for non-fertile eggs and treatment groups consisted of 30 eggs each. On the seventh day of the study, the blunt ends of the eggs (containing the air sacs) were cleaned with an appropriate disinfectant, a hole was made with the help of an egg pricker (Bai Shun, Zhejilang, China), and 50 µL of saline solution and drug applications were performed. The first group of the study was evaluated as a negative control, and no application was made.

The second group of the study was administered physiological saline in a volume of 50 µL; this was another control as the saline solution was the vehicle used as the carrier for the drug. The other five groups were administered Metronidazole (Fladazole 0.5% Solution for Injection, Istanbul, Türkiye) at a dose of 250, 125, 62.5, 31.2, and 15.6 µg·egg^{-1} (5, 2.5, 1.25, 0.625, 0.312 mg·kg^{-1}) within 50 µL saline. After these applications, the holes in the eggs were closed with liquid paraffin. Following treatment, no rotation was made for the first hour, but eggs were otherwise provided with optimum conditions in the incubator to ensure drug absorption from the air sac. At the end of the incubation period, the eggs hatched and embryotoxicity and teratogenicity rates were evaluated.

The actual mortality rate was determined using the Abbott formulation over the embryonic mortality rates [10, 11, 12]. Embryonic mortality rates between groups were evaluated using the Chi-square test (SPSS 22.2, IMD SPSS, Armonk, USA). A value of 0.001 was accepted as the threshold for statistical significance in the tests.

RESULTS AND DISCUSSION

The embryonic death rates following Metronidazole administration to fertile eggs are presented in TABLE I. No anomalies were detected in the macroscopic morphology of the embryos.

Metronidazole, a drug defined as category B by the Food and Drug Administration (FDA) in pregnancy, is an effective drug against anaerobic bacteria and some protozonoal infections that can cause embryotoxic or congenital malformations [1, 2, 3, 17, 18].

The drug was injected into the fertile chicken eggs on the seventh day when the liver detoxification mechanism was functional, in a volume of 50 µL saline, into the air chambers located at the blunt end of the eggs. Air chambers are preferred because of their advantages, such as low infection risk compared to other regions, rapid diffusion of the test solution, minimal mechanical damage compared to other regions, and ease of application [14, 19]. With these applications, Metronidazole's possible embryotoxic and/or teratogenic effects could be observed and measured.

TABLE I
Death rates from Metronidazole administration.

	Doses (µg·egg^{-1})	NAE	NDE	N	Death rate* (%)	Survival rate %	Actual death rate (Abbott method)
Chest-1	Control	28	2	30	6.7	93.3	–
	Saline control	29	1	30	3.3	96.7	–
	250	28	2	30	6.7	93.3	3.44
	125	29	1	30	3.3	96.7	0
	62.5	30	0	30	0	100	-3.44
	31.25	29	1	30	3.3	96.7	0
	15.62	29	1	30	3.3	96.7	0

NAE: Number of alive embryos, NDE: Number of dead embryos. *No statistical difference was determined in death rates (P>0.05)

This study found no statistical difference between the experimental groups (P>0.05, TABLE I). In addition, no macroscopic anomaly was observed in any group that received Metronidazole. Previous work has shown that teratogenic effects cannot be observed when Metronidazole is administered intragastrically at a daily dose of 2 mg·kg^{-1} during organogenesis in pregnant mice (*Mus musculus*) [20]. It is stated that as a result of controlled studies in pregnant mice, rats and rabbits, no teratogenic effects of Metronidazole were observed [21]. It is stated that fetal and obstetrical side effects are not observed when Metronidazole is administered at a dose of 500 mg·kg^{-1} twice a day or at a dose of 250 mg·kg^{-1} three times a day for seven days to pregnant women with genital system infection [22]. In humans, it was reported that 597 pregnant women (62 in the first trimester, 284 in the second trimester, and 251 in the third trimester) with trichomoniasis were treated at a dose of 200 mg three times per day for 7–10 days; no differences in prematurity, birth weight, or teratogenicity were found [23].

Neither was any congenital malformation observed in the babies of women who were administered Metronidazole at a dose of 200 mg three times per day for seven days at different times of pregnancy [24]. Two meta-analyses also reported no relationship between the teratogenic effects of Metronidazole use in the first trimester of pregnancy [25, 26]. Based on these studies, it has been stated that Metronidazole administered to treat trichomoniasis in women does not harm the fetus in the first trimester of pregnancy. However, it is still recommended to delay the treatment with Metronidazole until the second trimester [27].

It has also been reported that Metronidazole and Miconazole did not cause axial skeletal disorders in mice when either Metronidazole or Miconazole was administered at a dose of 60 mg·kg^{-1} intraperitoneally on the 8th, 9th, and 10th days of pregnancy. However, when these drugs were administered in combination, as is often done in the treatment of vaginal infections, there were teratogenic effects in mice; thus, there is potential for such effects also in pregnant humans [28]. In a study in mice, it was reported that when Metronidazole and ethyl alcohol were administered simultaneously, ethyl alcohol caused an increase in embryotoxicity and teratogenicity of Metronidazole [29]. When the literature reviews are evaluated together with the study data, it can be stated that Metronidazole alone does not cause lethal or morphological changes directly in the embryo.

CONCLUSION AND IMPLICATIONS

As a result, it can be stated that Metronidazole does not have any embryotoxicity and teratogenicity effects at the treatment doses it was used here. Therefore, its use during pregnancy can be safe. Due to the interaction of Metronidazole with many drugs, combined drugs are not recommended, especially during pregnancy. It can be suggested that this study should be supported by studies investigating the efficacy of the drug, with histopathological and/or molecular analyses on organogenesis especially in mammalian embryos.

ACKNOWLEDGEMENT

No financial support was received from any institution or organization for this study. We would like to thank Prof. Dr. Enver Yazar for his academic contributions.

Conflict of interest statement

The authors declare there is no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Freeman CD, Klutman NE, Lamp KC. Metronidazole : a therapeutic review and update. Drugs. [Internet]. 1997; 54:679–708. doi: https://doi.org/c55f26

[2] Ceruelos AH, Romero-Quezada L, Ledezma JR, Contreras LL. Therapeutic uses of Metronidazole and its side effects: an update. Eur. Rev. Med. Pharmacol. Sci. [Internet]. 2019; 23(1):397–401. doi: https://doi.org/gsbcbt

[3] Yazar E. Chemotherapeutics. In: Yazar E, editor. Veterinary Medicines Guide and Treatment Manual. Ankara, Türkiye: Atlas Yayınevi; 2023; p 79–236.

[4] Yazar E. Veterinary Drug and Vaccine From A to Z. Istanbul, Türkiye: Nobel Tıp Press; 2018; p 56–156.

[5] Dingsdag SA, Hunter N. Metronidazole : an update on metabolism, structure–cytotoxicity and resistance mechanisms. J. Antimicrob. Chemother. [Internet]. 2018; 73(2):265–79. doi: https://doi.org/gcg9fr

[6] Eren F, Aldan MA, Dogan VB, Gül G, Selcuk HH, Soysal A. A case with reversible neurotoxicity induced by Metronidazole. Ideggyogy. Sz. [Internet]. 2017; 70(11–12):429–432. doi: https://doi.org/k5s4

[7] Quickfall D, Daneman N, Dmytriw AA, Juurlink DN. Metronidazole –induced neurotoxicity. Can. Med. Assoc. J. [Internet]. 2021; 193(42):E1630. doi: https://doi.org/k5s5

[8] Dobiáš L, Černá M, Rössner P, Šrám R. Genotoxicity and carcinogenicity of Metronidazole . Mutat. Res. – Genet. Toxicol. [Internet]. 1994; 317(3):177–94. doi: https://doi.org/fxbksk

[9] Jelinek R, Peterka M, Rychter Z. Chick Embryotoxicity Screening Test–130 Substances Tested. Indian J. Exp. Biol. 1985; 23(10):588–595.

[10] Canbar R, Uslu M, Arslan MS, Kızılay H. Determination of Embryotoxic effects of Atipamezole using in ovo model. Rev. Cientif. FCV–LUZ. [Internet]. 2023; 33(1):1–4. doi: https://doi.org/k5xh

[11] Uslu M, Canbar R, Akcakavak G, Yazar E. Determination of the embryotoxic effect of maropitant using an in ovo model. Pol. J. Vet. Sci. [Internet]. 2022; 25(2):357–9. doi: https://doi.org/j2p7

[12] Canbar R, Akcakavak G, Uslu M, Bas AL. Determination of embryotoxic effects of Tarantula cubensis alcoholic extract with in ovo model. Magy. Allatorvosok Lapja. 2021; 143(8):497–504.

[13] Canbar R, Uslu M, Tufan Ö, Yazar E. Determination of embryotoxic dose limit of detomidine with in–ovo model. Eurasian J. Vet. Sci. [Internet]. 2021; 37(2):71–75. doi: https://doi.org/gkjqqc

[14] Ozparlak H. [The use of chick embryos in embryotoxicity and teratogenicity tests]. Selcuk Univ. Fen Fak. Fen Derg. [Internet]. 2015 [cited 25 Jun 2023]; 40:13–22. Turkish. Available in: https://bit.ly/3R9D9mh.

[15] Jelinek R. Use of chick embryo in screening for embryotoxicity. Teratog. Carcinog. Mutagen. [Internet]. 1982; 2(3-4):255–261. doi: https://doi.org/ch6ffn

[16] Hill E, Hoffman D. Avian models for toxicity testing. J. Am. Coll. Toxicol. [Internet]. 1984; 3(6):357–376. doi: https://doi.org/bc244f

[17] Nwosu OC, Bloom K. The safety of metronidazole in pregnancy. Health Care Women Int. [Internet]. 2021; 42(4–6):726–738. doi: https://doi.org/k5xk

[18] Rebuelto M, Loza ME. Antibiotic treatment of dogs and cats during pregnancy. Vet. Med. Int. [Internet]. 2010; 2010:1–8. doi: https://doi.org/dz75sd

[19] Hamilton JW, Denison MS, Bloom SE. Development of basal and induced aryl hydrocarbon (benzo [a] pyrene) hydroxylase activity in the chicken embryo in ovo. Proc. the National Academy Sci. [Internet]. 1983; 80(11):3372–6. doi: https://doi.org/d2jk7m

[20] Chacko M, Bhide S. Carcinogenicity, perinatal carcinogenicity and teratogenicity of low dose Metronidazole (MNZ) in Swiss mice. J. Cancer Res. Clin. Oncol. [Internet]. 1986; 112:135–140. doi: https://doi.org/dvzcw8

[21] Struthers BJ. Metronidazole appears not to be a human teratogen: review of literature. Infect. Dis. Obstet. Gynecol. [Internet]. 1997; 5(5):326–335. doi: https://doi.org/cwkq56

[22] Sobel R, Sobel JD. Metronidazole for the treatment of vaginal infections. Expert Opin. Pharmacother. [Internet]. 2015; 16(7):1109–1115. doi: https://doi.org/gq94jd

[23] Morgan I. Metronidazole treatment in pregnancy. Int. J. Gynaecol. Obstet. [Internet]. 1978; 15(6):501–512. doi: https://doi.org/k5xn

[24] Rodin P, Hass G. Metronidazole and pregnancy. Br. J. Vener. Dis. [Internet]. 1966; 42(3):210. doi: https://doi.org/bnsp3m

[25] Caro-Patón T, Carvajal A, Martín de Diego I, Martín-Arias LH, Alvarez–Requejo A, Pinilla ER. Is Metronidazole teratogenic? A meta-analysis. Br. J. Clin. Pharmacol. [Internet]. 1997; 44(2):179–182. doi: https://doi.org/dhqdk9

[26] Burtin P, Taddio A, Ariburnu O, Einarson TR, Koren G. Safety of Metronidazole in pregnancy: a meta–analysis. Am. J. Obstet. Gynecol. [Internet]. 1995; 172(2):525–529. doi: https://doi.org/fdnnzb

[27] Roe JFC. A critical appraisal of the toxicology of Metronidazole. In: Phillips I, Collier J, editors. Metronidazole. Proceedings of the 2nd International Symposium on Anaerobic Infection. Geneva, April 1979. New York: Grune and Stratton, 1979: 215–222.

[28] Tiboni GM, Marotta F, Castigliego AP. Teratogenic effects in mouse fetuses subjected to the concurrent in utero exposure to miconazole and metronidazole. Reprod. Toxicol. [Internet]. 2008; 26(3–4):254–261. doi: https://doi.org/fft2jk

[29] Damjanov I. Metronidazole and alcohol in pregnancy. J. Am. Med. Assoc. [Internet]. 1986; 256(4):472. doi: https://doi.org/cm5s9s