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Short communication

Determination of the embryotoxic effect of maropitant using an in ovo model

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Abstract

The aim of this research was to determine the embryotoxic and teratogenic effects and lethal dose (LD50) of maropitant in ovo, using fertile chicken eggs. The study was designed in two stages, CHEST-I and CHEST-II. For CHEST-I, 210 fertile eggs were divided into seven equal groups; control, saline solution and 5 different doses of maropitant (10, 5, 2.5, 1.25, 0.625 mg/kg) injected groups. For CHEST-II, 150 fertile eggs were divided into five equal groups; control, saline solution and 3 different doses of maropitant (8, 6, 4 mg/kg)-injected groups. Eggs were opened on day 21 of incubation. Maropitant did not cause teratogenicity at any dose, while higher embryonic death rates were observed at doses above 4 mg/kg. The LD_{50} dose of maropitant was determined as 7.24 mg/kg. In conclusion, maropitant should only be used after full consideration of risks and benefits in pregnancy.

Key words: Maropitant, embryotoxicity, in ovo model

Introduction

Maropitant, a neurokinin 1 receptor antagonist, is an antiemetic drug produced for dogs and cats. The drug is administered at doses of 1-2 mg/kg. However, its use during lactation and pregnancy is not recommended. As no reproductive toxicity studies have been conducted in target animals, maropitant should be used only in conjunction with benefit-risk assessment (Yazar 2018, EMA 2021). Fertile chicken eggs can be used instead of mammals in embryotoxicity and teratogenicity studies to examine the effects of chemical agents (Jelinek et al. 1985, Canbar et al. 2021). The Chick Embryotoxicity Screening Test (CHEST) was developed for this purpose in 1977 (Ozparlak 2015). Possible negative aspects of this method include the lack of maternal–fetal relationship, the possibility of false positive results due to high sensitivity, and the difference in pharmacokinetics in the closed system of the egg. However, embryotoxic dose levels obtained from the CHEST method reportedly provide predictable results that can be extrapolated to mammals.

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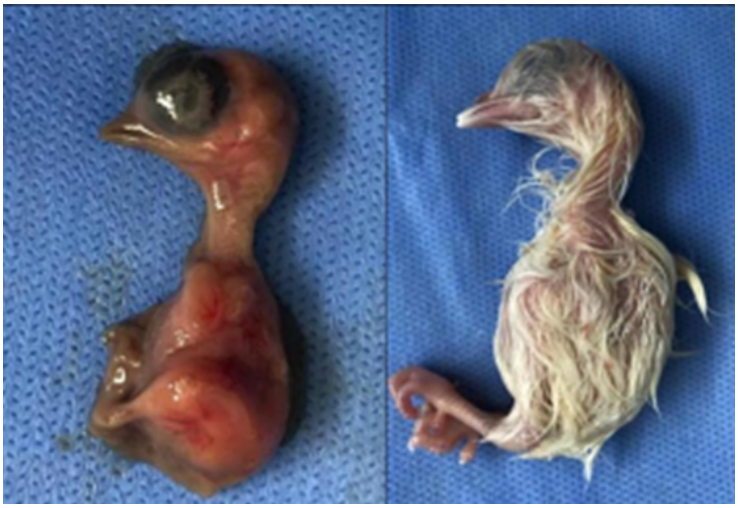

Fig. 1. Early (left) and late (right) embryonic death.

Table 1. Embryonic death rates in fertile eggs treated with maropitant (Chest-I and Chest-II).

	Groups	NVE	NDE	n	% Dead	% Alive	AM
Chest-I	10 mg/kg	4	26	30	86.66[a]	13.34	85.15
	5 mg/kg	21	9	30	30.00[b]	70.00	22.22
	2.5 mg/kg	28	2	30	6.66[c]	93.34	-3.70
	1.25 mg/kg	28	2	30	6.66[c]	93.34	-3.70
	0.625 mg/kg	28	2	30	6.66[c]	93.34	-3.70
	Saline solution	27	3	30	10.00[bc]	90.00	--
	Control	27	3	30	10.00[bc]	90.00	--
Chest-II	8 mg/kg	12	18	30	60.00[a]	30.00	58.62
	6 mg/kg	17	13	30	43.33[a]	56.67	41.37
	4 mg/kg	28	2	30	6.66[b]	93.33	3.44
	Saline solution	29	1	30	3.33[b]	96.67	--
	Control	29	1	30	3.33[b]	96.67	--

NVE: Number of viable embryos, NDE: Number of dead embryos, AM: Actual mortality (Abbott method).
[a, b, c]: Different letters in the same column are statistically significant (P<0.05).

In addition, the need for simple laboratory equipment, ease of application, rapid results, low cost, and the similarities in morphogenetic development in all living organisms have been reported as positive aspects (Jelinek 1982, Hill and Hoffman 1984, Ozparlak 2015). The CHEST test is evaluated in two stages; while CHEST-I results are used to determine the embryotoxicity limit, the CHEST-II stage is used to determine the teratogenicity parameters, by repeating the test three or four doses below the dose determined in the first stage (Jelinek et al. 1985, Ozparlak 2015).

Considering that chick embryos have been used previously instead of mammals (Jelinek et al. 1985, Canbar et al. 2021) in teratogenicity and embryotoxicity studies applied to mammalian pregnancies, we hypothesized that the possible teratogenic and embryotoxic effects of maropitant could be determined using fertile chicken eggs. This study aimed to determine the lethal dose (LD 50) of maropitant as well as its potential embryotoxic and teratogenic effects, using an in ovo model.

Materials and Methods

The research protocol was approved by the ethics committee (SUVDAMEK: 2021/100). This study used 360 fertile chicken eggs (Anatolian Integrated Breeder Animal Ltd, Konya, Turkey); Eggs were divided into twelve equal groups (n = 30) as CHEST-I (7 groups) and CHEST-II (5 groups), and incubated in an incubator (Imza Teknik, Konya, Turkey). For CHEST-I stage, 210 fertile eggs were divided into seven equal groups: Control, saline solution, and maropitant injected groups (10, 5, 2.5, 1.25 and 0.625 mg/kg). For CHEST-II stage, 150 fertile eggs were divided into five equal groups: Control, saline solution, and maropitant injected groups

(8, 6 and 4 mg/kg). The doses of maropitant were determined according to CHEST-I results. All injections were administered via the air sacs of the eggs on day 7 of incubation and maropitant (Cerenia™ injection, Zoetis, Istanbul, Turkey) was diluted in normal saline solution; total volume for injection was 50 µL. Eggs were opened on day 21 of incubation.

Actual mortality rates were determined using the Abbott method, based on observed mortality rates. Comparisons of embryonic mortality rates between groups were evaluated using the Chi-square test. LD_{50} doses of maropitant were calculated using the Probit test without converting to logarithms. This test was carried out using the combined CHEST-I and CHEST-II results (SPSS 22.0); a level of 0.05 was accepted as statistically significant.

Results and Discussion

In the current study, combined data from CHEST-I and CHEST-II stages revealed increased embryonic mortality rates in groups treated with 5-10 mg/kg maropitant (Table 1, Fig. 1). However, teratogenicity (wry neck, micromelia, etc.) was not observed at any dosage. Maropitant has no embryotoxic/fetotoxic effects in rats after oral administration of 15 mg/kg dose (EMA 2021). As the safety of maropitant has not been investigated in healthy pregnant dogs (El-Bahri 2009), it is recommended that it should only be used in pregnant or lactating bitches following a benefit–risk assessment (EMA 2021). Although there is no research available on the effects of maropitant during pregnancy, there have been limited studies on other NK-1 receptor antagonists such as rolapitant and aprepitant. Rolapitant did not produce teratogenic or embryo–fetal effects in rats and rabbits when administered orally during of organogenesis period at doses up to 1.2-2.9 times, respectively, the maximum recommended human dose (FDA 2015). However, the safety of rolapitant in pregnant women has not been assessed, and it has been reported to cause dose-dependent embryonic death and malformations in embryonated chicken eggs (Singh et al. 2021). Aprepitant is accepted as category B in human medicine, with no teratogenicity reported in animal studies (Cada et al. 2003); however, there are no reports of systematic testing in pregnant women (La Russo et al. 2008). In contrast, casopitant is not recommended for use in pregnant women (EMA 2009).

The embryonic LD_{50} dose of maropitant was determined as 7.24 (6.54-7.98) mg/kg according to the Probit test. Taking into account the injection volume used in the current study, maropitant could be administered at a maximum dose of 10 mg/kg. Results obtained from CHEST-I can be adapted to pregnant mammals; the value (mg/kg) obtained from CHEST-I multiplied by 10^{-2} is accepted as the toxic level for mammalian pregnancies (Ozparlak 2015).

Conclusions

Although teratogenicity has not been observed in rodents using an oral dose of 15 mg/kg, using an in ovo model, we have determined the dose of maropitant resulting in embryonic death to be >4 mg/kg. Considering the usual treatment doses of maropitant (1 mg/kg SC, 2 mg/kg PO), this drug can be accepted as safe in pregnant target animals at the recommended doses. However, maropitant should be used in conjunction with benefit–risk analysis in dog and cats.

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References

Cada DJ, Levien T, Baker DE (**2003**) Aprepitant. Hosp Pharm 38: 763-774.

Canbar R, Uslu M, Tufan O, Yazar E (**2021**) Determination of embryotoxic dose limit of detomidine with in-ovo model. Eurasian J Vet Sci 37: 71-75.

El-Bahri L (**2009**) Maropitant: A novel treatment for acute vomiting in dogs. Vet Med 104: 422-423.

EMA (**2009**) Casopitant https://www.ema.europa.eu/en/documents/withdrawal-report/withdrawal-assessment-report-zunrisa_en.pdf.

EMA (**2021**) Maropitant. https://www.ema.europa.eu/en/documents/scientific-discussion/cerenia-epar-scientific-discussion_en.pdf.

FDA (**2015**) Rolapitant. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/206500s003,208399s002lbl.pdf.

Hill EF, Hoffman DJ (**1984**) Avian models for toxicity testing. J Am Coll Toxicol 3: 357-376.

Jelinek R (**1982**) Use of chick embryo in screening for embryotoxicity. Teratog Carcinog Mutagen 2: 255-261.

Jelinek R, Peterka M, Rychter Z (**1985**) Chick embryotoxicity screening test – 130 substances tested. Indian J Exp Biol 23: 588-595.

La Russo J, Waldman SA, Kraft WK (**2008**) Aprepitant for the prevention of nausea and vomiting associated with chemotherapy and postoperative recovery. Expert Rev Clin Pharmacol 1: 27-37.

Ozparlak H (**2015**) The use of chick embryos in embryotoxicity and teratogenicity tests. SUFEFD 40: 13-22.

Singh V, Yadav Y, Solanki S (**2021**) Rolapitant: effects of an antiemetic agent on the growth of developing chick embryo. Int J Med Res Health Sci 10: 103-110.

Yazar E (**2018**) Veterinary drug and vaccine from A to Z, 1st ed., Nobel press, Istanbul, pp 153-154.