https://doi.org/10.52973/rcfcv-e33230 Revista Científica, FCV-LUZ / Vol. XXXIII, rcfcv-e33230, 1 – 4

Determination of Embryotoxic effects of Atipamezole using *in ovo* model

Determinación de los efectos embriotóxicos del uso de Atipamezol en modelo *in ovo*

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ABSTRACT

Atipamezole is a specific α2-adrenergic receptor antagonist, and there exists insufficient information on its use during pregnancy. The aim of this study was to determine the embryotoxic activity of Atipamezole through an *in ovo* method. During the first stage of the study, 210 fertile eggs were divided into seven groups of 30 fertile eggs and placed in an incubator. On the seventh day of the first stage, no application was made to the control group. The second group was administered 50 microliters (µL) of saline solution, while the other groups were given Atipamezole at doses of 250, 125, 62.5, 31.25 and 15.62 micrograms·egg^{-1} (µg·egg^{-1}) in 50 µL saline solution. In the second stage, according to the embryotoxic dose range determined from the first stage, 150 fertile eggs were divided into five groups of 30 fertile eggs and placed in an incubator. On the seventh day of the second stage, no application was made to the control group. Fifty µL of saline solution was administered to the second group. The other groups were given Atipamezole at doses of 220, 190 and 160 µg·egg^{-1} in 50 µL saline solution. After the incubation period, the eggs hatched, and the embryonic mortality rates were calculated. The mortality rate was determined to be 39.3% at the highest dose (250 µg·egg^{-1} = 5 miligrams·kilograms^{-1} –mg·kg^{-1}–) (P<0.05), while the mortality rate at other doses was determined to be the same as the control group (P>0.05). In conclusion, it can be stated that the dose determined for Atipamezole in this study was very high compared to the recommended doses and it can be used in pregnancy as a benefit-loss calculation when necessary. However molecular or histopathological studies regarding the development of organ drafts are necessary to determine the safety of its use during pregnancy.

Key words: Atipamezole; embryotoxicity; *in ovo*

RESUMEN

El atipamezol es un antagonista específico de los receptores adrenérgicos α2 y no existe suficiente información sobre su uso durante el embarazo. El objetivo de este estudio fue determinar la actividad embriotóxica de atipamezol mediante un método *in ovo*. Durante la primera etapa del estudio, se dividieron 210 huevos fértiles en siete grupos de 30 huevos fértiles y se colocaron en una incubadora. Al séptimo día de la primera etapa no se realizó ninguna aplicación al grupo de control. Al segundo grupo se le administró 50 microlitros (µL) de solución salina, mientras que a los otros grupos se les administró atipamezol en dosis de 250; 125; 62,5; 31,25 y 15,62 microgramos·huevo^{-1} (µg·huevo^{-1}) en 50 µL de solución salina. En la segunda etapa, según el rango de dosis embriotóxica determinado a partir de la primera etapa, se dividieron 150 huevos fértiles en cinco grupos de 30 huevos fértiles y se colocaron en una incubadora. Al séptimo día de la segunda etapa, no se realizó ninguna aplicación al grupo de control. Se administraron 50 µL de solución salina al segundo grupo. Los otros grupos recibieron atipamezol en dosis de 220; 190 y 160 µg·huevo^{-1} en 50 µL de solución salina. Después del período de incubación, los huevos eclosionaron y se calcularon las tasas de mortalidad embrionaria. Se determinó que la tasa de mortalidad era del 39,3% con la dosis más alta (250 µg·huevo^{-1} = 5 miligramos·kilogramos^{-1} –mg·kg^{-1}–) (P<0,05), mientras que la tasa de mortalidad con otras dosis se determinó que era la misma que la del grupo de control (P>0,05). En conclusión, se puede afirmar que la dosis determinada de atipamezol en este estudio fue muy alta en comparación con las dosis recomendadas y se puede utilizar en el embarazo como un cálculo de pérdidas y ganancias cuando sea necesario. Sin embargo, los estudios moleculares o histopatológicos relacionados con el desarrollo de borradores de órganos son necesarios para determinar la seguridad de su uso durante el embarazo.

Palabras clave: Atipamezol; embriotoxicidad; *in ovo*

Received: 26/01/2023 Accepted: 02/03/2023 Published: 13/03/2023

INTRODUCTION

α2-adrenergic receptors are found in many tissues and organs such as the central nervous, cardiovascular and digestive systems [4]. Norepinephrine has certain regulatory effects on the central nervous system by binding to α2-adrenergic receptors [17, 19]. When agonists, such as Xylazine, Detomidine, Medetomidine and Dexmedetomidine, bind to the α2-adrenergic receptors in the central nervous system, the release of Norepinephrine is prevented and sympathetic tone is decreased but sedation and analgesia is increased [21, 25]. Clinically, it has been reported that α2-adrenergic receptor agonists slow the heart rate with long-term hypotension after hypertension and causes side effects such as decreased cardiac output, kidney and liver damage, shock and respiratory depression [9, 23]. Some studies revealed that the effects of α2 receptor agonists are similar to each other, but the duration of action varies depending on the dose [9]. Antagonists of this receptor, including Atipamezole, Yohimbine and Tolazoline can be used to reduce the frequency of side effects caused by α2-adrenergic receptor agonists in pets [23].

Atipamezole (4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole) is a specific α2-adrenergic receptor antagonist that rapidly reverses the undesirable side effects caused by α2-adrenergic receptor agonists during the sedation phase in the Veterinary field [10, 17]. In addition, one study reported that it has proved useful in the Veterinary field for Amitraz poisoning [18]; in a study conducted in dogs (*Canis lupus familiaris*), Atipamezole was reported to be successful for treating Amitraz poisoning when administered intramuscularly at a dose of 50 micrograms·kilograms^{-1} (μg·kg^{-1}) [13]. In another study conducted in alpine mountain goats (*Rupicapra rupicapra*), the optimal anesthetic dose was 2.6–3.6 miligrams· kilograms^{-1} (mg·kg^{-1}) Xylazine, and 0.26–0.36 mg·kg^{-1} Atipamezole was used to reverse the efficacy of Xylazine [8]. An investigation conducted in mice (*Macaca mulatta*) stated that the effects of ethanol could be antagonized to a large extent using Atipamezole [20]. In Atipamezole toxicity studies, the letal dose 50 (LD_{50}) was reported to be higher than 30 mg·kg^{-1} in genetically modified Naval Medical Research Institute (NMRI) mice and Sprague-Dawley rats (*Rattus*) for intravenous, subcutaneous and intraperitoneal exposures. While calculating the LD_{50} it was reported that heart and lung damage occurred in the dead animals. When a 100 mg dose is administered to humans, restlessness, shivering, coldness and hypersalivation are observed, and the amount of plasma Norepinephrine increases, causing an increase in systolic and diastolic blood pressure [17]. There is no information regarding the safety of Atipamezole in pregnancy in target species [25].

Poultry embryos are frequently preferred for the investigation of embryotoxic and teratogenic effects of drugs and chemicals [5, 6, 14, 24]. This methodology has the advantages of knowing the developmental stages of the chicken (*Gallus gallus domesticus*) embryo, simplify of application and providing cheap and reproducible results. With the use of high sample sizes of chicken embryos, it is statistically superior to the studies on mammalian species. Using this method can help guide future prenatal toxicity studies in mammals and minimizes the number of test subjects as well as the pain suffered by the subjects. As a result, ethical rules, legal restrictions and animal rights are not contradicted [16]. Disadvantages of the poultry embryo toxicity methodology as it relates to mammalian toxicity studies include its lack of a maternal–fetal relationship that is observed in mammals and the pharmacokinetic disparities observed with the differences in chicken eggs compared to mammalian embryos [15]. However, its positive aspects include the need for little laboratory equipment, simplify of application, short time of experimentation, low cost and the understanding that morphogenetic events are similar in all living things [12, 15].

One study indicated that medication should be administered to the eggs in the early embryonic period to determine the toxicological dose limits. However, if the teratological effects of the metabolites that occur from the drug metabolism in the liver are being investigated, exposure during later developmental periods, during which the liver and kidneys complete their development, are preferred [16]. The liver is formed by the fourth day in poultry embryos, and the induction of enzymes increases after the seventh day [11].

Although Atipamezole has been reported to be safe in pregnant cattle (*Bos taurus*) [2, 3] it has been determined by the manufacturing company that the information regarding that statement is insufficient [26].

In this study, it was hypothesized that the toxic effects of Atipamezole on embryos in the *in ovo* model are dose dependent. The aim of the study was to determine the possible embryotoxic effects of Aipamezole using an *in ovo* model.

MATERIALS AND METHODS

Fertile chicken eggs were obtained from a commercial enterprise (Anadolu Damizlik, Konya, Turkey). During the study, the incubation periods were completed in an egg incubator (Imza Teknik, Konya, Turkey). On the seventh day of incubation, fertility was checked under light, and non-fertile eggs were removed from the groups. Fertile eggs were added to replace the non-fertile eggs, and treatment groups were comprised 30 eggs each. A commercial formulation of Atipamezole (Antisedan™ inj, Zoetis, Istanbul, Turkey) was used in the study. All doses were applied at a volume of 50 microliters (μL). The study was planned and performed in two stages.

Experimental design and animal practices

Stage 1: Determination of embryotoxic dose limit

In this study, 210 fertile eggs were randomly divided into seven groups of 30 fertile eggs and placed in an incubator. On the seventh day of stage one, the first group was treated as the control group, and saline with no Atipamezole was applied to the second group, which served as the vehicle control. Groups 3–7 received Atipamezole at doses of 250, 125, 62.5, 31.25 and 15.62 μg·kg^{-1} (5, 2.5, 1.25, 0.625, 0.3125 mg·kg^{-1}) respectively**.** After the incubation period of 21 days, the eggs hatched, and the numbers of live and dead embryos were recorded (İmza Teknik, Konya, Turkey).

Stage 2: Determination of embryotoxicity

In the second stage, 150 fertile eggs were randomly divided into five groups of 30 fertile eggs and placed in an incubator. On the seventh day of stage two, the first group was treated as the control group, and saline with no Atipamezole was applied to the second group, which served as the vehicle control. Groups 3–5 received Atipamezole at doses of 220, 190 and 160 μg·egg^{-1}, respectively. These were within the embryotoxic dose limits determined from the first stage. After the incubation period of 21 days, the eggs hatched, and the numbers of live and dead embryos were recorded.

Statistics

Mortality rates were calculated using the Abbott method. Intergroup embryonic mortality rates were evaluated for differences using the Chi-square test (SPSS 22.2, IMD SPSS,Armonk, USA) [5].

RESULTS AND DISCUSSION

In this study, Atipamezole caused an observed death rate of 39.3% at a dose of 5 mg·kg^{-1} (TABLE I).

Atipamezole is a specific α2-adrenergic receptor antagonist that rapidly reverses the undesirable side effects caused by α2-adrenergic receptor agonists during the sedation phase in the veterinary field [10, 17]. There is insufficient information regarding the use of the drug during pregnancy [26].

In this study, it was determined that Atipamezole caused embryotoxic activity at a high dose (5 mg·kg^{-1}) at a rate of 39.3% (P<0.05), which was significantly different from the other treatment groups. No statistical differences were detected among the other treatment groups (P>0.05, TABLE I). The recommended application doses of Atipamezole are 0.03–0.04 mg·kg^{-1} in cats (*Felis catus*) and dogs and 0.03–0.06 mg·kg^{-1} in horses (*Equus caballus*) and cattle (*Bos taurus*) [25]. In a study conducted in humans, Atipamezole was administered at a dose of 0.05 mg·kg^{-1} against Dexmedetomidine [1]. In a different study, it was shown that Atipamezole did not exhibit negative effects on offspring and pregnancy at the 0.162 mg·kg^{-1}dose in two pregnant cattle who had been administered Medetomidine [3]. It was reported that the administration of Atipamezole at a 0.045 mg·kg^{-1}dose in cattle during the last two months of pregnancy did not have a negative effect on calves and that premature birth or periparturient disorders were not observed [2]. It was revealed that Atipamezole administered at a dose of 0.75 mg·kg^{-1} changed creatine kinase activity compared to non-pregnant females in a study conducted on pregnant reindeer.

TABLE I
Death rates from Atipamezole administration

	Doses (µg·egg^{-1})	NAE	NDE	N	Death rate (%)	Alive rate (%)	Actual death rate (Abbott method)
Chest-1	Control	28	2	30	6.7^{b}	93.3	–
	SF control	29	1	30	3.3^{b}	96.7	–
	250	17	13	30	43.3^{a}	56.7	39.3
	125	28	2	30	6.7^{b}	93.3	0
	62.5	29	1	30	3.3^{b}	96.7	–3.6
	31.25	28	2	30	6.7^{b}	93.3	0
	15.62	28	2	30	6.7^{b}	93.3	0
Chest-2	Control	29	1	30	3.3^{b}	96.7	0
	SF control	30	0	30	0^{b}	100	0
	220	28	2	30	6.7^{b}	93.3	3.5
	190	29	1	30	3.3^{b}	96.7	0
	160	29	1	30	3.3^{b}	96.7	0

NAE: Number of alive embryos, NDE: Number of dead embryos, $^{a, b}$: Different letters in the same column represent statistical significance (P<0.05)

Furthermore, abortion was not observed within 10–18 hours after application of Atipamezole at a 6.3 mg/goat dose in the transport of hook horned mountain goats [7, 22]. From this study, it should be considered that the Atipamezole was used at a dose of 5 mg·kg^{-1}, which is high compared to the recommended doses.

CONCLUSIONS

From this study, Atipamezole may be embryotoxic in high doses (5 mg·kg^{-1}). However, the drug efficacy of embryos on organ primordia has yet to be investigated. In future studies, it is suggested that researchers conduct molecular and histopathological studies on embryo development.

Ethical committee

The study was approved by the ethics committee of Selcuk University, Faculty of Veterinary Medicine, Experimental Animals Production and Research Center (SUVDAMEK) with the number 2021/35.

ACKNOWLEDGEMENTS

For this study, no financial support was received from any institution or company. The summary of the research was presented orally at the II International Veterinary Pharmacology and Toxicology Congress, 7–10 September, 2022, Balikesir University, Turkey and the summary was published in the booklet.

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