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Amitraz: pharmacological and toxicological aspects in animals

[*Amitraz: aspectos farmacológicos e toxicológicos em animais*]

"Revisão/Review"

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Abstract
Infestations by ectoparasites are one of the greatest problems in the veterinary practice, due to a large drop in performance in cattle herds and other farm animals, generating great economical losses; for pets, besides the low quality of life and disruption of homeostasis, ectoparasites represent a risk for transmission of zoonotic diseases. The formamidines emerge as a very large group of ectoparasiticides, which its main representative, amitraz, is the only one approved for animal use. Amitraz is indicated for animal use against mites, lice, and ticks for cattle, swine, and sheep. For dogs, it is used against ticks and mites. However, due to a lack of proper orientation and information, reports of accidental intoxications by amitraz, in both animals and humans, are not unusual in the literature. Amitraz intoxication has been reported in dogs, and the clinical signs are evidenced in the nervous, digestive, cardiovascular, and urinary systems and include sedation, bradycardia, bradyarrhythmias, hypotension, bradypnea, transitory hyperglycemia, mydryasis, and hypothermia, cats being more sensitive than dogs regarding these last signs. To detect amitraz and its main metabolites, there are many standardized methodologies. This review describes the pharmacokinetics, pharmacodinamics, indications, toxicological and pathological effects of amitraz, as well as the intoxication treatment and the aspects related to its detection in biological matrices.

Keywords: Formamidines; poisoning; dog; pathology.

Resumo
As infestações por ectoparasitas representam um dos maiores problemas na rotina da clínica médica veterinária, com impactos relacionados à queda no rendimento de animais de produção, gerando graves perdas econômicas; para os animais de companhia, além da baixa qualidade de vida e do desequilíbrio da homeostase, ectoparasitas representam um risco para a transmissão de zoonoses. As formamidinas surgem como um grupo de ectoparasiticidas cujo representante principal, o amitraz, é o único aprovado para uso animal. O amitraz é indicado para uso animal contra ácaros, piolhos e carrapatos de bovinos, suínos e ovinos. Para cães, preconiza-se seu uso contra carrapatos e ácaros. Entretanto, devido à ausência de orientação adequada, os casos de intoxicações acidentais por amitraz, tanto em animais como em humanos, são frequentemente relatados. A intoxicação por amitraz tem sido relatada em cães, e os sinais clínicos são evidenciados em sistemas nervoso, digestivo, cardiovascular e urinário e incluem sedação, bradicardia, bradiarritmias, hipotensão, bradpneia, hiperglicemia transitória, midríase e hipotermia, sendo os gatos mais sensíveis do que os cães em relação a estes últimos sinais. Para detectar amitraz e seus principais metabólitos, existem muitas metodologias padronizadas. Esta revisão descreve a farmacocinética, farmacodinâmica, indicações de uso, efeitos toxicológicos e patológicos, além do tratamento da intoxicação e dos aspectos analíticos relacionados à sua detecção em matrizes biológicas.

Palavras-chave: Formamidinas; envenenamento; cão; patologia.

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Introduction

Parasitism is one of the greatest problems in veterinary medicine, especially in hot and wet tropical countries (Sartor and Santarém, 2006). Ectoparasitism occupy a prominent place among the parasitic diseases that affect farm animals, raising direct and indirect costs, due to the drop of the animals' yield and the need for a sanitary control (Fonseca et al., 2009). Meanwhile, for companion animals, the problem is also severe, since it directly compromises their welfare, besides representing a risk to human health, due to zoonotic transmission (Dantas-Torres and Otranto, 2014).

In the 19th century, arsenical solutions were the first substances used for control of ectoparasites, and were introduced in Brazil by 1900. After the discovery of resistance mechanisms to ectoparasiticides, other compounds were developed, such as dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), toxaphene e dieldrin. From 1941, new acaricidal compounds have been synthesized, such as organophosphates, organochlorides, carbamates, and pyrethroids (Sartor and Santarém, 2006).

Chlordimeform, the first formamidine, appeared in 1963 (Hollingworth, 1976), followed by other drugs such as amitraz, or N'- (2,4-dimethylphenyl)-N-[(2,4-imethylphenyl) imino-methyl] -N-methylmehtanimidamide, of molecular formula C19H23N3 (Pubchem, 2015), in England in 1969 (Hollingworth, 1976). This is the most used active substance among the formamidines, including in Brazil, because it is the only one approved for animal use (Sakate et al., 1992; Sartor and Santarém, 2006).

This review describes the pharmacokinetics, pharmacodinamics, indications, toxicological and pathological effects of amitraz, as well as the intoxication treatment and the aspects related to its detection in biological matrices.

Pharmacokinetics and pharmacodinamics

Amitraz is quickly hydrolyzed in an acid environment when it is orally administrated, due to its instability in this environment (Sartor and Santarém, 2006). The hydrolysis in a low pH generates the compound 2,4-dimethylphenyl formamide, which is stable in an acid environment. This substance can still be hydrolyzed, generating 2,4-dimethylaniline (Pierpoint et al., 1997). Absorption is effective through the skin, which may be major or minor depending on its integrity, the occurrence of injuries, and inflammation. After reaching the blood stream, the drug reaches the highest plasmatic level in up to two hours (Dallegrave and Sebben, 2008). The biotransformation occurs in the liver, generating the active metabolite BTS 27271, the most important pharmacologically (Santarém et al., 2008), because it acts directly in the regulation of the insulin and glucagon secretion by binding to the α2A and α2D-adrenergic receptors, inhibiting insulin and stimulating glucagon secretion, resulting in hyperglycemia (Abu-Basha et al., 1999). Metabolites are excreted in bile and urine (Sartor and Santarém, 2006; Santarém et al., 2008).

In insects, formamidines such as chlordimeform and amitraz operate its toxic effects by interacting with octopaminergic receptors in the central nervous system (Evans and Gee, 1980; Chen et al., 2007). The mechanisms by which the deleterious effects of amitraz in mammals are determined are based on its agonistic action on α2-adrenergic receptors and inhibitory action over the monoamine oxidase (MAO), but there are reports of various action pathways, such as: H1 histamine receptor inhibition, prostaglandin synthase inhibition, adenylyl cyclase inhibition, voltage-gated calcium channels activation, reactive oxygen species generation, cell death induction and endocrine disruptions. Amitraz is also related to the emergence of neurotoxic effects and modifications in the reproductive sphere in rats (Del Pino et al., 2015).

Indications and contraindications

Amitraz is indicated for animal use against mites, lice and ticks for cattle, swine and sheep. For dogs, it is used against ticks and mite (Sartor and Santarém, 2006). It was reported as the drug of choice in the treatment of localized and generalized demodicosis in dogs (Folz et al., 1984). Along with macrocyclic lactones such as milbemycin oxime, ivermectin, moxidectin, and doramectin, amitraz is still recommended for the treatment of generalized canine demodicosis, although it is not very efficient in adult-onset demodicosis cases (Mueller, 2004; Mueller et al., 2012). Cowan and Campbell (1988) and Chesney (1989) also reported the use of amitraz in the treatment of demodicosis in cats. Gunaratnam et al. (1983) evaluated amitraz toxicity in cats, and concluded that low concentrations, around 0,0125% are capable of generating moderate toxic effects, especially

anorexia. The toxic effects are even more evident in cats, due to their licking habit, resulting in a higher intake of the product. However, it is possible to use amitraz topically in healthy cats, respecting the appropriate contraindications common to the other species, which is to avoid the use in diabetic, hypothermic, and cardiac patients (Andrade et al., 2007).

Amitraz is contraindicated in horses due to the risk of hypomotility and intestinal atony, leading to severe intestinal impaction (Duarte et al., 2003; Santarém et al., 2008). Duarte et al. (2003) observed, besides the intestinal symptoms, the occurrence of neurologic signs as drowsiness, decreased cranial nerve reflexes and ataxia in horses submitted to the experimental use of amitraz. This substance is contraindicated for patients with extended skin injuries, which could lead to an over absorption, favoring intoxication (Santarém et al., 2008).

Toxicological and pathological aspects of amitraz poisoning in animals

In Brazil, data provided by the Sistema Nacional de Informações Tóxico-Farmacológicas (SINITOX) showed that in the year of 2013, 42128 cases of human intoxications and 255 cases of animal intoxications of any etiologies were registered (Fiocruz, 2013a). Veterinary products were responsible for the notification of 307 cases of human intoxications and 7 cases of animal intoxications in the same year (Fiocruz, 2013b). However, these numbers may be even higher, due to a high rate of underreported cases by the Centro de Informação e Assistência Toxicológica (CIAT) of each Brazilian state (Fiocruz, 2013).

Several studies about amitraz acute toxicity were previously conducted in many species, regarding the median lethal dose 50 (LD50) (Table 1).

Table 1. Oral LD_{50} values for dog, mouse, rat and chick.

Species	LD_{50}	Authors
Mouse	>1600 mg/kg	Al-Thani et al. (2003)
Rat	515 - 938 mg/kg	Dallegrave and Sebben (2008)
Dog	100 mg/kg. Intoxication signs at doses of 20 mg/kg.	Dallegrave and Sebben (2008)
Chick	53,05 mg/kg	Al-Hammdani and Al-Baggou (2014)

Amitraz intoxication has been reported in dogs, and the clinical signs are evidenced in nervous, digestive, cardiovascular, and urinary systems (Oglesby et al., 2006) and include sedation, bradycardia, bradyarrhythmias, hypotension, bradypnea, transitory hyperglycemia, mydryasis, and hypothermia, cats being more sensitive than dogs regarding these last signs (Andrade et al, 2008). Emesis, diarrhea, abdominal pain, and intestinal hypomotility are observed in the digestive system. In the urinary system, there is polyuria (Xavier et al., 2008). It is the stimulation of α2-adrenergic receptors that generates the main signs of amitraz poisoning, such as loss of consciousness, breathing depression, seizures, bradycardia, hypotension, and hypothermia (Proudfoot, 2003).

Electrocardiographic changes have been reported after the experimental use of amitraz in healthy dogs. Right after the intravenous administration of amitraz, there was a reduction in the duration of the P wave and an increase in the length of QT, PT, and RR intervals, but without changes in the QRS complex and in the amplitude of the R wave, besides a marked bradycardia. These results are consistent with the α2-agonistic action of amitraz, but there were no sinoatrial blocks, as expected (Farias et al., 2005). In a different study, the cardiovascular and respiratory effects after the intravenous administration of amitraz in dogs, in increasing doses of 1, 2 and 5 mg/kg were reported. Thus, an increase in blood pressure during one hour and an initial bradycardia were observed, which returned to normal levels in a dose-dependent way. The tidal volume, the respiratory rate, and the respiratory volume per minute presented an initial drop. On the other hand, hyperventilation was observed after the use of high doses of amitraz (Cullen and Reynoldson, 1987).

Accidental poisoning with amitraz was described in two Simmental cows. The animals received accidental intramuscular injections of amitraz, and among the clinical signs, anorexia, depression, reduced rumen motility, congestion of episcleral vessels, light bloat, and loss of pupillary reflex were observed. Heart and respiratory rates and rectal temperature remained unchanged. The poisoning was treated with yohimbine, and after 24

hours, the animals were clinically stable (Kizil et al., 2008).

In a study on the acute intoxication by amitraz in mice, the histopathological findings after the oral administration of growing doses of both the technical and commercial formulations of amitraz in two distinct groups were analyzed. It was observed that the groups that received doses equal or higher than 1500 mg/kg of the technical formulation presented degenerative hepatic changes. At a dose of 1500 mg/kg, there was hydropic degeneration; at 1800 mg/kg, both hidropic and fatty degeneration; at 2200 mg/kg and 2500 mg/kg, there were severe degenerative injuries. Similar injuries were seen in animals who received the commercial formulation, but at a dose equal or higher than 250 mg/kg. This kind of formulation also led to the development of epithelial cell necrosis at a dose of 500 mg/kg, and at the doses of 750 mg/kg and 1000 mg/kg, tubular epithelial cells necrosis was observed (Filazi et al., 2004).

Omoja et al. (2016a) evaluated the hepatic and renal effects of the subchronical administration of a commercial formulation of amitraz 12.5% in albinoWistar rats for 84 days. Four experimental groups were designated, whose doses corresponded to 10 mg/kg, 2 mg/kg, and 0.4 mg/kg, and the fourth group received 10 ml/kg of water, by oral route. The group treated with 10 mg/kg presented moderate hepatic vacuolar degeneration of periportal hepatocytes and mild to moderate generalized tubular degeneration in kidneys, affecting pars recta, proximal convoluted tubules, and collecting tubules. The remaining groups didn't show any significant changes. This way, it can be inferred that the chronic use of amitraz in high doses may provoke severe damages to the hepatic and renal tissues. Through the same protocol, Omoja et al. (2017) also observed that rats exposed to a 10 mg/kg dose, given daily by oral route may present regenerative anemia by the 30th day of treatment, analyzed by the mean levels of packed cell volume, red blood cell and total white blood cell counts, and hemoglobin.

The amitraz toxicity related to the reproductive sphere was evaluated in rats, which received orally growing doses of the drug (40, 120, and 360 ppm). Significant changes were observed only after a 360 ppm dose, in which there was a decrease in the seminal vesicles' weight, decrease of sperm motility, fewer living offspring, and increased losses after deployment (Lim et al., 2010). Omoja et al. (2016b) also investigated the reproductive effects in male Wistar rats sub-chronically exposed to growing doses of amitraz by oral route, and concluded that there is a dose-dependent influence of amitraz over testosterone production and sperm reserve, besides the appearance of testicular degeneration in the group treated with a dose of 10 mg/kg/day.

In a case report of accidental poisoning with amitraz in a dog, which resulted in the death of the animal, the main post mortem findings were pulmonary edema, hemorrhagic gastroenteritis, cardiac, pulmonary, renal, hepatic and splenic hemorrhages, and also brain congestion and edema (Andrade et al., 2004).

In a second case of accidental poisoning in a dog by ingestion of a bait containing amitraz, the necropsy revealed severe ventral subcutaneous edema and moderate serosanguineous ascites, diffuse hemorrhage on the left kidney and partial on the right kidney. Histopathological analysis of the kidney showed severe diffuse cortical hemorrhage, obliterating the interstitial tissue; glomerular and tubular necroses were also evident, besides a neutrophilic infiltration due to the necrosis. The cells presented intense eosinophilia, unbundling of the basal membrane, karyolysis and pyknotic nuclei. Yet, there were certain multifocal deposits of fibrin, severe cortical vessel congestion and moderate congestion in the medulla. Histopathological analysis of the liver revealed severe congestion and sinusoidal enlargement, hepatocyte atrophy, multifocal fatty degeneration, and some necrotic areas (Oglesby et al., 2006).

Treatment

The reversion of amitraz poisoning in animals is based on the supportive therapy, gastrointestinal decontamination, and the use of α2-adrenoceptor antagonists such as yohimbine (Andrade et al., 2005; Smerdel et al., 2008) and atipamezole (Hugnet et al., 1996; Andrade et al., 2005). Yohimbine is reported for reversing the alterations caused by amitraz administration in high concentrations, such as hypotension, bradycardia, emesis, salivation, mydriasis, moderate sedation, hyperglycemia, and in some cases, synusal arrhythmia (Smerdel et al., 2008). Atipamezole is a potent selective α2-adrenoceptor antagonist, not interacting with other receptors. It is a great antagonist of xylazine and medetomidine,

drugs known for their agonistic activity on α2-adrenergic receptors, as is amitraz (Cardoso et al., 2011).

Laboratory analyses

To detect amitraz and its main metabolites, there are many standardized methodologies. According to Hugnet et al. (1996), it is possible to evaluate the plasmatic toxicokinetics of amitraz in dogs by high-performance thin layer chromatography (HPTLC), an easily reproducible technique. Zanella et al. (1999) quantified amitraz in ectoparasiticide bath solutions used in cattle by HPLC-UV technique. This technique was effective for detection of amitraz in the samples, and has allowed quantifying the active ingredient without any interference from foreign substances. Saito et al. (2008) validated a methodology for detection of amitraz and its metabolites in human serum by liquid chromatography-mass spectrometry (LC-MS), using monolithic silica column with acetonitrile. Marafon et al. (2010) seek to standardize and validate the technique of gas chromatography with thermionic sensitive detection (GC-TSD), and thus determine the amitraz concentration in the blood of cats experimentally submitted to ectoparasiticide dipping baths of amitraz 0.4%. This technique comes in use to determine the pharmacokinectics of amitraz in cats. GUO et al. (2014) developped an analitical methodology for amitraz and its metabolites N-[2,4-(dimethylphenyl) -N'-methyl-formamidine, 2,4-dimethylformamidine e 2,4-dimethylaniline in human blood samples, by solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry. This methodology was effective, sensitive, selective, and reproducible to detect and analyze amitraz and its metabolites in blood samples.

Conclusion

Due to its high efficacy against a wide range of ectoparasites in livestock and pet animals, amitraz is a common product in veterinary practices and homes. As a result, both accidental and intentional poisoning in animals and humans is currently underreported and requires wider attention from both medical and veterinary practitioners.

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