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Pharmacokinetic Characteristics of the Drug Based on Moxidectin for Young Stock and Small Breed of Domestic Animals

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ABSTRACT

The pharmacokinetic characteristics of moxidectin in the blood serum of dogs and cats after a single cutaneous (spot-on) application of drug for veterinary use "Inspector Mini" to prevent and treat arachnoses, entomoses and intestinal nematodes in kittens and puppies as well as in small breed dogs and cats were investigated. Twelve outbred dogs and cats of different ages and weights were involved in present study. All the animals were weighed to determine the exact dosage of the drug. The determination of moxidectin in blood serum was carried out by high performance liquid chromatography with pre-column modification of N-methylimidazole and trifluoroacetic anhydride followed by fluorescence detection. According to the results of the study, it was found that moxidectin was well absorbed into the systemic circulation and reached to maximum concentration in the blood serum of dogs and cats after 4-10 days. After treatment with the drug, moxidectin was determined in the blood serum of animals after 12 hours at concentration of 2 ng/ml. Significant concentrations of moxidectin in the blood serum of animals remained for 28 days after topical application (spot-on). Moxidectin was detected in the blood serum of animals at the end of the experiment (after 30 days) which indicates its therapeutic effect for at least one month after the application of the drug.

Key words: Blood Serum, Cats, Dogs, Moxidectin, Pharmacokinetics

INTRODUCTION

The main methods against parasites of carnivores are preventive treatment of animals for which more and more new drug are constantly being developed (Arisov et al., 2018; Arisova et al., 2019). However, it should be noted that most of these drugs have limitations for especial ages and weights. In this regard, small breed dogs and cats and as well puppies and kittens with a body weight of less than 1 kg remain unprotected from parasites. Taking into account the urgency of this problem, the drug names "Inspector Mini" was developed for veterinary use to treat small breed dogs, cats, puppies and kittens against ectoparasites, nematodes and dirofilariasis as well. The drug combination contains 5 mg/ml of moxidectin as an active substance. Moxidectin is a semisynthetic compound of the milbemycin group (macrocyclic lactones) has a profound systemic and contact effect against the larvae and adults of ectoparasites and nematodes, has a stimulating effect on gamma-aminobutyric acid release, increases the permeability of membranes for chlorine ions which inhibits the activity of parasitic nervous cells causing a disorder of muscle innervation, paralysis and death of ectoparasites and nematodes. Pharmacokinetics and metabolism of moxidectin was studied in rats, sheep and cattle. According to the data of scientific research, moxidectin has a higher intrinsic potency against some parasites, especially filarial nematodes, than the avermectins. It has also high distribution in lipid tissues, poor metabolism and a long half-life. So that effective concentrations of moxidectin persist for longer time in target hosts, which makes it possible to have a low resistance to parasites. Moreover, moxidectin has a high safety index (Prichard and Geary, 2019). The compound Moxidectin is mostly released intactly from the body. The therapeutic efficacy of macrocyclic lactones in arachnoses and entomoses is high (Nolan and Lok, 2012; Balandina, 2017; Bespalova et al., 2018). The drug is used for animals by the drip (spot-on) application on a dry intact skin in places out of reach of licking (between shoulder blades at the base of the neck at the following doses (Table 1).

The drug is used in a dose of 0.2 ml per 1 kg of animal weight during treatment. Safety of the drug for use in animals at recommended dosage regimen was confirmed during studying its tolerability. According to the extent of exposure, "Inspector Mini" belongs to low-hazard substances (class 4 hazard according to GOST 12.1.007-76), so it does not have a local irritation, resorptive-toxic and sensitizing effect at the recommended doses (Simaeva at al., 2017).

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Table 1. Dose of Inspector Mini according to body weight of dogs and cats

Animal species	Animal mass (kg)	Dose of the drug (ml)	AI concentration(mg/kg)
Dogs, puppies	0.5 – 2.0	0.4	1 – 4
Cats, kittens	0.5 – 2.0	0.4	1 – 4

MATERIALS AND METHODS

Six outbred dogs weighted 2.6-4.5 kg and aged 8-14 months along with six outbred cats weighted 1.0-1.9 kg and aged 6-9 months were used in the experiment. These animals were kept in nurseries with no history of chemotherapy and health problems before the investigation (30 days). For accurate dosing of the drug all animals were weighed. The drug was applied to the animals once by the spot-on method at the rate of 1 mg moxidectin per 1 kg of body weight of dog or cat. Blood sampling was performed prior to the study (0 h) and 12, 24, 72, 120, 168, 240, 360, 480, 600 and 720 hours after application of the drug. The blood samples were collected in polymer tubes without an anticoagulant and blood serums were separated and frozen up to the beginning of the study. Moxidectin in the blood serums of animals was determined by HPLC on Shimadzu Prominence LC20 chromatograph with RF-20Axs fluorimetric detector. Quantitative determination was carried out with the external standard method. The procedure of calibrating chromatographic data was used to carry out a qualitative and quantitative analysis of the obtained blood serum extracts (liquid extraction with hexane). The calibration procedure has two goals: determination of the retention time of the analyzed component for its following identification (qualitative analysis of samples) and determination of the analyte concentration with a calibration curve (external standard method). The example of the chromatogram of blood serum extract of moxidectin standard solution obtained by HPLC is presented in figure 1.

The calibrating procedure on moxidectin in the eluent solution was repeated twice in different days. For calibration procedure, solutions with concentrations of 100, 50, 10, 5, 1 ng/ml were used. For calibration procedure on serum samples, solutions of moxidectin with concentrations of 1, 5, 10, 50 and 100 ng/ml were used. The average extraction rate of moxidectin from blood serum was 79%. Metrological approval of test procedure on the content of moxidectin in the blood serum was carried out in accordance with Hartmann et al. (1998), Rockville (2000), Chiap et al. (2003), Epstein (2004), Senyuva and Gilbert (2011) and Prichard and Barwick (2012). Several blood serum solutions (samples for calibration) with concentrations of 1, 5, 10, 50, and 100 ng/ml were used for the experiment. The obtained data are presented in tables 2 and 3.

Thus, the proposed method was linear in the range of 1-100 ng/ml (R>0.99) and showed good precision and accuracy. The method makes it possible to identify moxidectin in the model and blood serum samples. The pharmacokinetic parameters were calculated with PK Solver program (Zhang et al., 2010).

Ethical approval

The most humane and operational methods and manipulations were used in the study; immediate measures to prevent pain and distress in dogs and cats. All painful manipulations with the bird were carried out in accordance with regulatory standards: European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS No. 123; Strasbourg, 18 March 1986), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Strasbourg, 22 September, 2010).

Table 2. Linearity and parameters of the test method for blood serum

Limit of detection (LOD), (ng/m)	Limit of quantification (LOQ), (ng/m)	Calibration curve	Linearity range, (ng/ml)
0.29	0.97	y = 11165 * R > 0.999	1 – 100

LOQ: limit of quantification, LOD: limit of detection.

Table 3. Parameters of precision and accuracy of the method

Matrix	Measuring range, (ng/ml)	RSD (%)	Δ (%)
Blood serum	1.0 – 5.0	7.7	37.4
	5.0 – 100.0	6.9	12.4

RSD: Relative Standard Deviation, Δ: accuracy factor, LOD: Limit of detection

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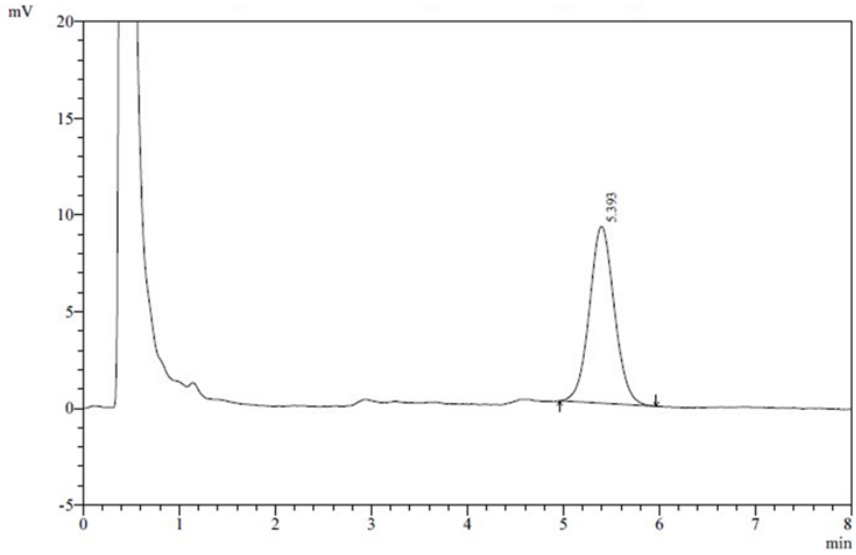

Figure 1. Chromatogram of the blood serum extract of moxidectin standard solution (10 ng/ml). Derivative of moxidectin in 5.4 minute is presented in figure

RESULTS

As a result of studying the pharmacokinetic characteristics of the drug after spot-on application to dogs and cats, moxidectin was determined in the blood serums of animals after 12 hours at a concentration about 2 ng/ml. The maximum concentration of moxidectin in the blood serum of dogs reached 3.8 ng/ml on the third day of the study and remained at roughly the same level (3.6 ng/ml) up to the seventh day. Then there was a gradual decrease in the concentration of the active substance to 1.3-1.7 ng/ml in the blood serums by 30 days after a single dose of the drug "Inspector Mini" which is used in veterinary (Figure 2).

The studies of the pharmacokinetics of moxidectin in cats showed that the maximum concentration of active ingredient also reached 5.3 ng/ml on the third day of the study and remained at the level of 5.3-5.1 ng/ml up to 7 days after treatment. Then the moxidectin concentration gradually decreased to 1.5-3.5 ng/ml by 30 days of the study (Figure 3). The calculation of pharmacokinetic parameters of moxidectin in the blood of dogs and cats are presented in table 4. The obtained data showed that moxidectin is absorbed into the blood in both species used in present study. The active ingredient was found in the blood serum of dogs and cats at the end of the experiment (30th day) which indicated its therapeutic effect for at least 1 month after application.

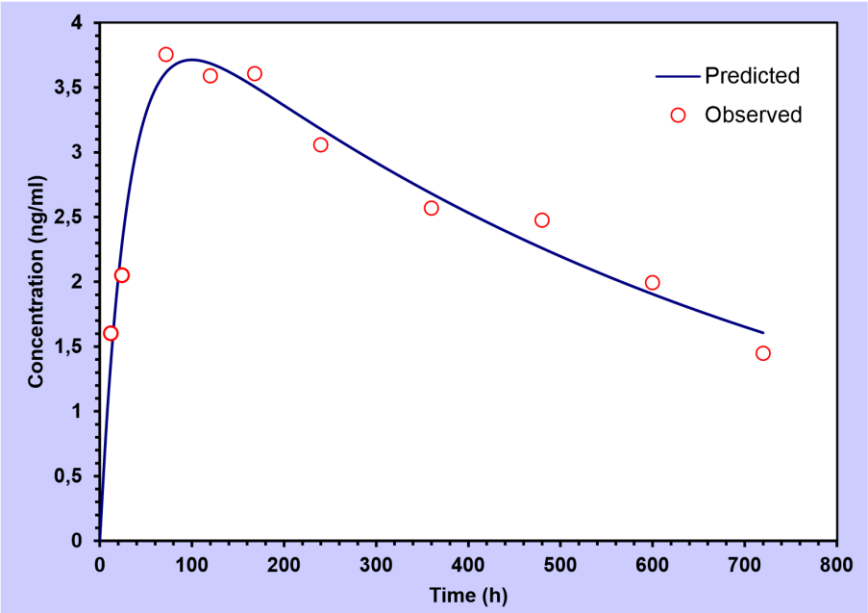

Figure 2. Concentration-time pattern of moxidectin in the blood serum of dogs

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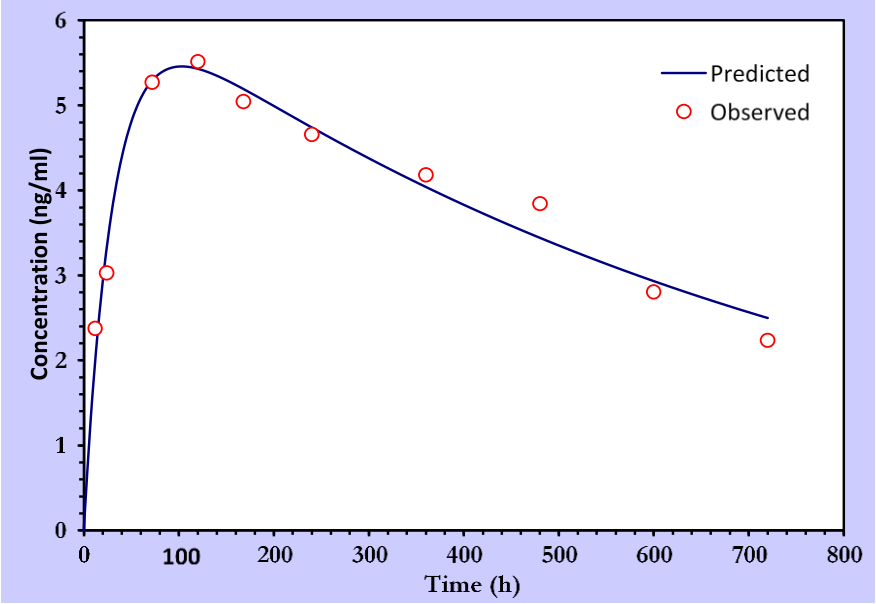

Figure 3. Concentration-time pattern of moxidectin in the blood serum of cats

Table 4. Pharmacokinetic parameters of moxidectin in the blood of dogs and cats

Parameter	Pharmacokinetic parameters of moxidectin in the blood of dogs		Pharmacokinetic parameters of moxidectin in the blood of cats	
	Average	RSD (%)	Average	RSD (%)
K_l, h^{-1}	3.83×10^{-2}	54.7	3.82×10^{-2}	53.8
K_{el}, h^{-1}	1.60×10^{-3}	44.1	1.29×10^{-3}	46.4
T_{half}, h	504	39.6	617	36.7
V_d, ml/kg body weight	246805	41.4	170868	39.7
Cl, ml/kg body weight/h	337	4.7	196.2	24.3
T_{max}, h	104	40.8	112.4	44.6
C_{max}, ng/ml	3.8	31.2	5.5	27.8
AUC $_{(0\text{-}Inf)}$, ng/ml*h	1884	24.2	2936	24.7
AUC $_{(0\text{-}T)}$, ng/ml*h	2970	4.7	5357	24.4
AUMC $_{(0\text{-}Inf)}$, ng/ml*h^2	2262839	33.2	5126399	52.3
MRT, h	766	34.7	929	32.9

RSD: Relative Standard Deviation, K_l: Langmuir constant (absorption rate of AI in injection site), K_{el}: elimination constant (elimination rate), T_{half}: elimination half-life of drug substance, V_d: apparent volume of distribution, Cl: Clearance (drug clearance from the system), T_{max}: time-to-peak concentration of drug substance, C_{max}: value of maximum concentration of the drug substance, AUC: Area Under Curve(area under curve of the drug substance in blood), AUMC: Area Under Multiplication Curve(area under the first moment versus time curve), MRT: Mean Residence Time.

DISCUSSION

The present study showed that the dynamics of distribution and elimination of moxidectin in the body of these two species of carnivores is approximately the same although the serum concentrations of the studied compound were higher in cats. High values of the area under the curve and the volume of distribution indicate the accumulation of moxidectin in tissues, particularly in the subcutaneous fat layer, which provides drug activity against ectoparasites. It should be noted that the long-term prolongation of moxidectin (high values of AUC) in the blood of dogs and cats confirms the preventive effect of "Inspector Mini" against cardiac dirofilariasis. The calculated elimination half-life and MRT values prove that the moxidectin circulates in the animal body for a long time. Comparison of the pharmacokinetics of the combination of Inspector Quadro (fipronil, pyriproxifen, praziquantel and moxidectin) revealed the presence of moxidectin in the blood serum of cats within 1 month and in dogs - within 40 days after a single dose application (spot-on) in identified concentrations (Arisov et al., 2018; Arisova et al., 2019). This drug is intended for the treatment and prevention of endo- and ectoparasites in dogs and cats from 1 kg and at least 7 weeks old according to the instruction for use. In the pharmacokinetic studies of the complex antiparasitic drug «Gelminthal» (syrup), moxidectin remained in the cats and dogs' blood for a long time (up to 30 days) which indicates its therapeutic effect for at least 1 month after use (Arisov et al., 2016a). These findings suggest the use of the drug based on moxidectin to prevent dirofilariasis in

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domestic animals. The relevant studies of moxidectin of Gelminthal tablets found that the pharmacokinetics of this substance are almost the same in dogs and cats which had good distribution in tissues and preservation in serum samples up to 25 days (Arisov et al., 2016b). The Contraindication of Gelminthal tablets is for animals with body weight less than 0.5 kg. The results of the pharmacokinetics study of moxidectin by other researchers showed that it is rapidly absorbed through the skin and is slowly removed (Xiao et al., 2019). The skin half-life of moxidectin is longer, potentially covering the entire mite life cycle (Bernigaud et al., 2016), which is important for the effective treatment against ectoparasites.

When comparing the comparative plasma dispositions of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following subcutaneous administration in rabbits, it was found that moxidectin was absorbed faster from the injection site and reached to the peak in plasma concentration (C_{max}) significantly earlier than IVM and DRM. The mean plasma residence time and terminal half-life ($t_{1/2\lambda z}$) were longer for DRM and MXD compared with IVM (Gokbulut 2010). This finding indicated that the persistence of moxidectin are significantly long and it has a positive impact on its efficacy against parasites or utility relating to interdosing interval. There is a scientific evidence that the complex antiparasitic drug "Inspector Total" (fipronil and moxidectin), which is used for skin administration in dogs and cats, protects animals from 14 types of parasites and prevents from reinfestation for up to 2 months (Gavrilova 2013). But this drug is not used for puppies and kittens under 7 weeks old and the treatment of dogs and cats under one kg is carried out carefully under veterinarian supervision. It should be noted that complex drugs might not always be suitable for young animals due to the toxicity of any active component. Thus, the developed new drug based on moxidectin "Inspector Mini" for dermal administration is an alternative to protect young stock and small breed domestic animals from helminths and ectoparasites.

CONCLUSION

As a result of pharmacokinetic studies of moxidectin in the blood serum of dogs and cats, it was found that the active ingredient of the drug for veterinary use the Inspector Mini was absorbed into the blood of carnivores after spot-on application and was determined within 30 days, thereby providing the necessary therapeutic and preventative effect against nematodes and ectoparasites.

DECLARATIONS

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Authors' contribution

Irina P. Belykh designed the study, interpreted the data, and drafted the manuscript, carried out the research work, compared, analyzed data and critical checking of this manuscript and also final acknowledgement of the version to be published.

Competing interests

Author state no conflict of interest.

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