Food and Chemical Toxicology 105 (2017) 319–321

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short communication

Relationship between ivermectin concentrations at the injection site, muscle and fat of steers treated with traditional and long-acting preparations

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ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form
1 May 2017
Accepted 3 May 2017
Available online 4 May 2017

Keywords:
Ivermectin
Injection site
Muscle
Fat
Cattle
Residues

ABSTRACT

Ivermectin (IVM) is broad-spectrum compound active against endo and ecto-parasites of clinical relevance in veterinary and human medicine. It is commercially available to use in livestock animals as injectable formulations containing 1% IVM and also as a concentrated (3.15%) long-acting (LA) preparation. The potential risk of the presence of high concentrations of drug residues in muscle tissue at the injection site is therefore a concern. The aim of this study was to determine the relationship between the IVM residual concentrations at the injection site, in comparison to the untreated contralateral neck (control muscle) and in fat from cattle treated with different preparations. Healthy steers received one of the following subcutaneous treatments in the neck area: Group A: IVM-LA 3.15% preparation and Group B: IVM 1%. After a withdrawal period for each formulation the animals were sent to the slaughterhouse. IVM concentrations at the injection site were detected among animals treated with the LA formulation, with high residual concentrations of IVM (between 15 and 141 μg/kg) in the injection site of two steers. However, the residual concentrations of IVM at the injection site obtained after the subcutaneous administration of the LA preparation do not represent a toxicological risk to consumers.

1. Introduction

Ivermectin (IVM) is a semi-synthetic avermectin broad-spectrum compound active against endo- and ecto-parasites of clinical relevance in veterinary and human medicine. IVM is commercially available to use in livestock animals as either injectable, oral or pour-on formulations. Injectable formulations containing 1% IVM are preparations that yield a slow absorption process from the subcutaneous space, resulting in an extended persistence of IVM concentration in the bloodstream, and in tissues where parasites are located. Ivermectin is a fat-soluble drug that is widely distributed by the animal's circulation due to its slow release from fatty tissue, which acts as a reservoir site (Chiu et al., 1986; Lanusse et al., 1997). A highly concentrated (3.15%) long-acting (LA) IVM preparation to be administered to cattle at 630 μg/kg was developed to extend the antiparasitic persistency period with a single treatment (Lifschitz et al., 1999, 2007). It is recommended that subcutaneous administration of injectable IVM formulations is in front or behind the shoulder. The administration of veterinary drugs such as anthelmintics may develop lesions in the musculature adjacent to the region of subcutaneous injection (Mann et al., 2011). Besides, the potential risk of the presence of high concentrations of drug residues in muscle tissue at the injection site is therefore a concern. For the evaluation and detection of residual concentrations of IVM, regulatory agencies specify the liver, kidney, muscle and fat tissues as target tissues for analysis. Different criteria are adopted by various regulatory agencies on whether the injection site should be treated in the same way as other tissues with regards to residue repletion. The aim of this study was to determine the relationship between the residual concentrations at the injection site, in comparison to the untreated contralateral neck (control muscle) and in fat from cattle treated with traditional and LA formulations of IVM.

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http://dx.doi.org/10.1016/j.fct.2017.05.005
0278-6915/

2. Materials and methods

2.1. Experimental animals, treatments and sampling

Eight healthy 20-month-old Hereford cattle were selected from a ranch neighboring Tandil, in the Province of Buenos Aires, Argentina, and identified with ear tags. The animals were in optimal nutritional condition and had free access to food and water during the entire experimental period. The experimental animals were weighed before treatment (360 ± 58 kg). Individual injectable doses were calculated based on the weight of the experimental animals and the IVM concentration of the preparations used in the current trial. Animals in each group received one of the following subcutaneous treatments in the neck area:Group A: IVM-LA 3.15% preparation (Ivomec Gold®, Merial) injected at the recommended dose of 630 μg/kg at day −122, and Group B: IVM 1% (Ivomec®, Merial) given at the recommended 200 μg/kg dose rate at day −37. The injection site was marked on each animal in order to follow it throughout the study up to sampling post-mortem. After the proscribed withdrawal period for each formulation (120 and 35 days, respectively) the animals were sent to the slaughterhouse with an average liveweight of 420 ± 42 kg. Muscle samples from the marked injection site, samples from the same neck muscle on the contralateral untreated side (control muscle samples) and abdominal fat samples were taken. To ensure that the collected tissues encompass the injection site, a core of muscle tissue (15 cm of diameter and 2.5 cm deep, weighing approx. 180 g) was taken. The collection of the injection site muscle tissue was centered on the marked point of injection. The muscle and fat samples were ground and then stored in vials at −20 °C until analysis.

2.2. Analytical procedures

2.2.1. Preparation of samples for HPLC

A muscle sub-sample (0.5 g) was taken after mincing and homogenizing the entire area of the site of injection (180 ± 10 g) and was placed into a plastic tube and spiked with 10 ng of the internal standard (IS) abamectin (10ng/10 μl). Similarly, 0.5 g of each fat sample was placed in a tube and spiked with the IS. The samples were kept at room temperature for 1 h. Three replicates were analyzed for each experimental sample. IVM and abamectin pure reference standards were obtained from Sigma Chemical Co (St. Louis, MO, USA). Stock solutions (1 mg/ml) and serial dilutions were prepared in methanol. The extraction of IVM from different tissues, the derivatization process and the HPLC analysis were carried out following the technique described by Lifschitz et al. (1999 and 2000). Briefly, drug molecules were extracted by the addition of 1 ml of acetonitrile and after mixing in a high-speed vortexing shaker, the sample was sonicated and centrifuged at 2000*g* for 10 min at 4 °C. The supernatant was transferred to a tube and the extraction procedure repeated. Water (a volume equal to that of acetonitrile) was added to the pooled supernatants. The total was transferred to C18 cartridges (Strata, Phenomenex, CA, USA) using a manifold vacuum. Drug were eluted with 1.5 ml methanol. The elution was evaporated and the dry residues were dissolved in 100 μl of *N*-methylimidazole solution in acetonitrile (1:1, v/v) and 150 μl of trifluoroacetic anhydride (1:2, v/v). IVM was analyzed in muscle and fat samples by HPLC with fluorescence detection (Shimadzu LC-20A HPLC system, Kyoto, Japan) fitted with a Kromasil C18 (5 mm, 250 × 4.60 mm) reverse-phase column (Eka Chemicals, NY, USA) at 30 °C and a fluorescence detector (Shimadzu; RF 10A XL detector) with 365 nm excitation and reading at 475 nm emission. The mobile phase consisted of acetic acid (0.2% in water, v/v), methanol, and acetonitrile (5:40:55 v/v/v), and was pumped at a flow rate of 1.5 ml/min.

A complete validation of the analytical procedures for the extraction and quantification of IVM in the matrices was performed before the analysis of experimental samples. Calibration curves in the range of 0.2–150 μg/kg were plotted using the peak-area ratios between IVM and the IS. The data were analyzed for linearity using a least-squares linear regression analysis and using ANOVA to determine whether the data differed from a straight line. The absolute recovery, inter-day precision, accuracy and limit of quantification were defined for each matrix. Drug concentrations in experimental samples were determined from the HPLC results by calculating the ratio between the areas under the peaks of IVM and the IS using the CR10 software and interpolating these areas on the calibration lines prepared for each matrix. The statistical program (Instat 3.0; Graph Pad Software Inc., San Diego, CA, USA) was used for linear regression analyses and linearity tests.

3. Results

The validity of the method for quantification of IVM by HPLC was proven. The linear regression lines for IVM showed correlation coefficients >0.99. The mean recoveries of IVM were >70%. The precision of the analytical procedures obtained after HPLC analysis of IVM showed a CV < 10%. The limit of quantification was established at 0.2 μg/kg.

Ivermectin was detected in the muscle of injection site after the treatment with the LA formulation. Significant differences in IVM concentrations at the injection site were detected among animals treated with the LA formulation. Residual concentrations of IVM (between 15 and 141 μg/kg) were detected at the injection site of two steers, whereas negligible amounts of drug were detected in the injection sites of the other two animals treated with the LA formulation. European Medicines Agency (EMA) and the Joint Expert Committee on Food Additives (JECFA) established a maximum residues limit for muscle of 30 μg/kg (EMA, 2014; WHO, 2015). No residual concentrations of IVM were detected in the muscle samples taken from the contralateral untreated neck (control sample) and in the fat of animals treated with LA formulation.

No concentrations of IVM were detected at the injection site, the muscle sample taken from the contralateral untreated neck or the fat of animals treated with the traditional 1% IVM formulation. The IVM residual concentrations in the different tissues obtained after the administration of both formulations are summarized in Table 1.

4. Discussion

The introduction of the LA formulation into the veterinary pharmaceutical market was addressed to extend the drug action

Table 1
Ivermectin residual concentrations (μg/kg) in steers obtained in different tissues at day 37 and 122 after the administration of either 1% and 3.15% formulation respectively.

Animal number	Injection site	Muscle of contralateral untreated site	Fat
Ivermectin 3.15%			
060	ND	ND	ND
728	141.6 ± 38.7	ND	ND
004	15.7 ± 7.89	ND	ND
781	ND	ND	ND
Ivermectin 1%			
008	ND	ND	ND
633	ND	ND	ND
079	ND	ND	ND
637	ND	ND	ND

ND: no detected.

against different pathogens. The extended persistence of the drug in the body determines the long withdrawal period for the anthelmintic LA formulations. Whereas the traditional IVM preparation is approved with a withdrawal time of 35 days, the period for the LA formulation is 120 days, based on the evaluation of the main target tissues such as liver, kidney fat and muscle. In the current trial, there were no residual concentrations of IVM in a marker tissue (fat) nor in the contralateral muscle of the neck from the injection site after the administration of both formulations under study. These results indicate that the systemic IVM concentrations on day 37 and 122 after the administration of the traditional and LA preparation, respectively, are very low, and consequently the drug level in the target tissues is undetectable. Although the established withdrawal times provide assurance that foodstuffs obtained from animals treated with these preparations will not contain residues, the drug-related residues at the injection site demand additional consideration.

Residual concentrations of IVM were detected at the injection site of two of the four animals treated with the LA formulation (Table 1). The detection of residual drug concentrations at the injection site is very variable and may be caused by the incomplete absorption of the total administered volume. The lesions that different vehicles may produce at the injection site (George et al., 1995) may contribute to the persistence of a low proportion of the dose in the administration area. Although most of the lesions occur after intramuscular injection, the subcutaneous administration may cause some muscle damage (Van Donkersgoed et al., 2000). After the subcutaneous treatment, the solvent of the formulation or the precipitation of the drug may irritate the local tissue (Rasmussen, 1980; Reeves, 2007). Among the different preparations available in the veterinary pharmaceutical market, the sustained-release injectable formulations, (e.g. non aqueous vehicles, such as propylene glycol, and various oils) irritate tissues after the subcutaneous injection (Baggot and Brown, 1998). In the current trial, the subcutaneous injection of the LA formulation produced a small area of lesion at the injection site in both animals in which IVM concentrations were detected.

From the view of human food safety, an important concern is the slow depletion of residues of some injectable formulations from the site of administration. Some regulatory agencies recommend that injection site residues in food producing species should be treated in the same way as the other edible tissues with regard to residue depletion. However, as the residues at injection sites tend to deplete erratically and more slowly than in other tissues (Nouws et al., 1990), the application of the maximum residue limit for muscle also to the muscles at the injection site may lead to an increased withdrawal time. On the other hand, it was clearly demonstrated that the risk of injection site consumption is more related to an acute exposure risk than to a chronic one, which is usually posed by the residues present in other edible tissues (Sanquer et al., 2006). Clinical studies demonstrate that a 15 mg oral dose of IVM is well tolerated. If a ten-fold safety factor is applied to this 15 mg oral dose and a consumption of 500 g of meat from the injection is considered, a safe concentration limit of 3000 μg/kg at injection sites could be established (FDA, 1995). The maximum concentration of IVM seen in this study at the injection site was 141 μg/kg, so that residual concentrations detected in two of the animals treated with the LA formulation are lower than those indicated in the evaluation of food security given above. Although the European Union assumes that the consumers may be chronically exposed to residues at the injection sites, an injection site residue reference value of 1250 μg/kg was recently proposed (EMA, 2014). Other countries such as the USA, Canada and Australia recommend the acute reference dose method, assuming that the injection site residues are consumed infrequently (Sanquer et al., 2006). A combined analysis of different studies performed by the JECFA proposed a Global Estimate of Acute Dietary Exposure (GEADE) of 52 μg/kg bw for the general population (WHO, 2015).

An international harmonization related to risk analysis of residues at the injection site is necessary to ensure food safety and to avoid problems in the international meat trade (Reeves, 2007). The data obtained in this study confirm that the rational use of veterinary drugs that respects the withdrawal times indicated for each formulation is essential for food security of consumers. The results of this study indicate that residual concentrations of IVM at the injection site of cattle obtained after the subcutaneous administration of the LA preparation do not represent a toxicological risk to consumers.

Acknowledgments

The current work was supported by CONICET, Agencia Nacional de Promoción Científica y Técnica and Instituto de la Promoción de la Carne Vacuna Argentina (IPCVA).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.05.005.

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