EFFECT OF FORMULATION IN LIVER AND MUSCLE RESIDUE LEVELS FOR IVERMECTINE

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Background

Ivermectin is a fermentation derived antiparasitic agent having broad spectrum endo and ecto parasitic activity in animals. It is the 22,23-dihydro derivative of avermectin B_1, a macrocyclic lactone produced by an actinomycetes, *Streptomyces avermitilis* . It consists of two closely related homologues containing no less than 80 % 22,23-dihydroavermectin B_{1a} (H_2B_{1a}) and no more than 20% 22,23-dihydroavermectin B_{1b} (H_2B_{1b}).(Campbell,1993)
It is well-known that the vehicle or excipient in drug formulation may have an important role in the process of drug-absorption, plasma concentration profile and in consequence, residue depletion profile (Steel,1993). Studies made with ivermectin have shown that the pharmacokinetics and hence efficacy of this compound can be sustancially modified by formulation (Lo & Williams,1985). Others investigators have shown that the antihemintic efficacy of this drug in cattle is associated with prolonged plasma concentrations (Bogan & Mc Kellar, 1988). But also, the influence of the formulation must be examined with emphasis on their impact on tissue residues. Pharmacology and tissue depletion studies of ivermectin in its clasic non acqueous formulation have been reported by several authors (Lanusse at al., 1997; Chiu et al.,1986; FAO-WHO,1993), the unaltered drug was the major residue in food tissues, being liver and fat the target tissues (those were residues are larger).Nowadays, new oil-based formulations of ivermectin are being developed to prolonge its antiparasitic action by reducing the absorption from the injection site and increasing in this way the persistence in plasma. The effect of the formulation in plasma kinetic profile and its consequence in residue profile must be investigated .

Objectives

Following the development of a new injectable oil-based formulation for cattle, the aim of this study was to evaluate the phamacokinetic profile and the residue depletion in liver and muscle to assure the safety of meat consumption.

Methods

Six male Hereford calves were used, two non treated calves were used as controls.The animals were injected with a new formulation containing 3.15 % of ivermectin with 1.5 % of castor oil. The dose employed was 50 ml/kg. Blood samples were taken at 0, 8, 16, 24 h and at 2, 3, 4, 7, 17, 28, 38, 56, 73, 93 and 109 days post-treatment. Tissue samples were taken by biopsy at 7, 28, 38 and 56 days post-treatment for liver and at 7, 17, 28, 38, 56, 73, 93 and 109 days for muscle. Blood samples were centrifuged and recovered plasma kept at -18 °C until analysis. Tissue samples were stored at the same temperature.
Plasma samples were extracted by liquid-solid methodology and determined by HPLC with fluorescent detection (Montigny et al., 1990). Samples of bovine tissues were homogenized by using a blender and assayed in triplicate. A 1g amount of C_{18} material was weighed into a mortar and the tissue sample (0.25g) was added. For recovery studies, 10 µl of endectocides solutions at 0.5 µg/µl were injected into the tissue 5 min before the extraction. The extraction and cleaned-up procedure is based on the matrix solid phase dispersion technique (MSPD) and was achieved by using a previously described process (Alvinerie et al., 1996).
Calibration graphs were constructed using the peak area as a function of analyte concentration and least-squares regression analysis was used to determine slope. The correlation coefficient generally exceeded 0.990. The limits of quantification (LOQ) were 0.09 and 0.36 ppb for plasma and tissues respectively, being the limits of detection (LOD) 0.05 and 0.2 ppb. The extraction recoveries were 83,6 % (CV%= 11,3%), 88,7% (CV= 8,0%) and 87,9 % (CV=11,5%) for plasma, liver and muscle respectively.

Results and discussion

Ivermectin was detected in plasma between 8h and 109 days post-administration indicating a prolonged residence in the bloodstream. Plasma depletion for the mean values is presented in figure 1. Maximun plasma concentration is attained 4 days post-treatment indicating a quickly absorption of the drug. Plasma levels drops between 7 and 28 days following a linear logarithmic kinetic as if the drug was distributed and eliminated at a constant rate (figure 2). Plasma concentrations are constants until 73 days post-treatment and finally descend slowly, being still quantified at 109 days post-treatment. This prolonged persistence of the drug in the plasma indicates that it is still being absorbed and distributed, thus balancing its elimination. After 73 days post-treatment, plasma levels declines indicating the prevalence of the elimination process.
This experiment shows the influence of the formulation in the pharmacokinetic profile. The most striking difference between the clasic (Lanusse et al.,1997)and this oil-based formulation of ivermectine is the long persistence of the drug in plasma. This finding is consistent with the hypothesis of more persistent parasitic drug exposure due to an absorption retarded in time.
Muscle and liver depletion of ivermectin residues are presented in figures 3 and 4. Liver residues are higher than muscle residues at all times post-treatment. These results are in agreement with those reported for the clasic formulation of the drug, but there is an important

difference concerning the levels observed. Liver residues are similar to those obtained for the clasic formulation at 7 days p.t., but they shows a more attenuated slope at higher times post-treatmentindicating that an important quantity of drug is been metabolized. Muscle residues for the clasic formulation are very low , the maximun level reachs 15 ppb at 8 days post-treatment and are undetectable at 38 days pot-treatment. For the oil-based formulation, muscle residues reach 54ppb at 7 days post-treatment, drops quickly until 38 days and then slowly until 109 days p.t., following a kinetic similar to plasma concentrations. The comparative kinetics are shown in figure 6. As it has been suggested before analysing plasma results, the absorption, distribution and biotransformation of the drug is still taking place according to these results.

These results can have an important consequence for the meat safety from animals treated with an oil based formulation. For the clasic formulation, residue levels of treated animals are below the maximun limit of residues (LMR) fixed by Codex Alimentarius at 18 days p.t. in liver (LMR=100 ppb) and 28 days p.t. for muscle (LMR=2ppb), being the liver usually used as target tissue in residue control. In this experience, liver residues are at the LMR at 38 days p.t. and muscle ones at 93 days p.t., suggesting that the use of liver as control tissue is questionable.

Conclusions

Oil-based formulation used to prolonge parasitic action strongly changes the plasma pharmacokinetic profile and the residue distribution and depletion in food tissues. There are a longer persistence in plasma and higher concentrations in tissues, particularly in muscle. In this last tissue, the time to reach the LMR are much more longer than in liver, usually the target residue control tissue. This considerable difference provides evidence for suggesting that the residue control for animals treated with the oil based formulation must be reconsiderated.

References

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