and ABZSO are debated in the literature. The aim of this study was to investigate the genotoxic effect of ABZSO related to plasma concentrations in sheep lymphocytes.

MATERIALS AND METHODS

ABZSO was administered at 5 mg kg^{-1} body weight subcutaneously to eight sheep. Heparinised blood samples were collected from the jugular vein before drug administration as well as at different times between 2 and 96 h after drug administration. The plasma concentrations of ABZSO in plasma were analysed by high performance liquid chromatography following solid phase extraction procedures. The genotoxic effect of ABZSO was evaluated by Comet assay (1) in sheep lymphocytes. To this end, a suspension of sheep lymphocytes was mixed with low melting agarose and layered onto slides precoated with agarose. Lymphocytes were lysed to liberate the DNA. Then, electrophoresis was carried out under highly alkaline conditions. After neutralization, DNA stained with fluorescent ethidium bromide. Zero hour sheep lymphocytes were treated with 50 and 100 μM H_2O_2 as positive control cells. Quantitative extent of DNA damage (mean tail moment) was determined in 100 randomly selected lymphocytes by image analysis software linked to a camera attached on a fluorescent microscope.

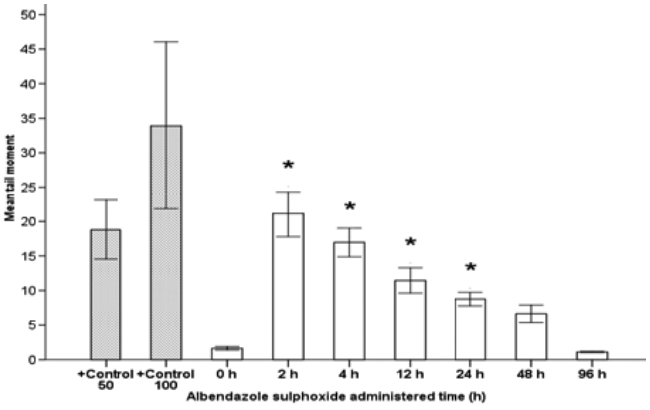

Figure 1. Evaluation of albendazole sulphoxide genotoxicity on sheep lymphocytes after 5 mg kg^{-1} body weight subcuteneous administration. *$P < 0.05$ versus 0 or 96 h.

RESULTS

Plasma levels of ABZSO at 0, 2, 4, 12, 24, 48, 96 h were 0, 1.33 ± 0.33, 1.71 ± 0.37, 1.73 ± 0.32, 0.75 ± 0.34, 0.03 ± 0.01 and 0 μg ml^{-1}, respectively. Comet assay results showed a significant decrease of DNA damage between 2 and 48 hours after ABZSO treatment (Fig. 1). The sheep lymphocyte DNA damage was significantly higher at 2, 4, 12 and 24h compared to control values (100 μM H_2O_2; $p < 0.05$) ($P < 0.05$). Positive correlation between the sheep lymphocyte DNA damage and plasma concentrations of ABZSO was observed ($r_s = 0.727$, $P < 0.001$). DNA damage returned to basal levels 48 h after the ABZSO treatment.

CONCLUSIONS

There is debate on the potential genotoxic, mutagenic or carcinogenic effects of albendazole treatment in the literature. Various authors state that benzimidazole derivatives, such as for example, albendazole, were suspected of clastogenic, teratogenic or cytotoxic activity (2–6). Previously, the micronucleus test proved that albendazole treatment increased the frequency of micronuclei. The treatment of sheep with albendazole initiated disbalance of the oxidative – antioxidative equilibrium as a result of oxidative stress. This study showed that a subcutaneous dose of ABZSO at 5 mg kg^{-1} body weight produced DNA damage in circulating lymphocytes. Although, this damage seems to be repaired within 48 h, in concordance with ABZSO plasma levels. However, a positive correlation with ABZSO plasma levels and DNA damage ($r_s = 0.727$, $P < 0.001$) indicated the influence of other factors. This study presented the apparent genotoxic effect with the Comet assay and would suggest follow-up analyses.

REFERENCES

1. Collins (2011). Methods in Molecular Biology, 691.
2. Dayan (2003) *Acta Tropica* 86: 141–159.
3. Dimitrijević *et al.* (2012). *Vet Parasitology* 186: 364–375.
4. Mattson *et al.* (2012) *Reproduction Toxicology* (in press).
5. Oztas et al. (2007). International Journal Infectious Diseases 11: 446–449.

2.2.53
Comparative hepatic metabolism of the anthelmintic flubendazole in rat, swine and sheep

G. VIRKEL, M. L. MATÉ, A. LIFSCHITZ, L. CEBALLOS, L. I. ALVAREZ & C. E. LANUSSE
FCV-UNCPBA, CIVETAN-CONICET, Tandil, Tandil, Argentina

INTRODUCTION

Flubendazole (FLBZ) is a broad-spectrum benzimidazole anthelmintic widely used in pigs and poultry. This drug has shown good efficacy to control gastrointestinal nematodes in sheep and macrofilaria in lymphatic filariasis and onchocerciasis in man (Mackenzie & Geary, 2011). Flubendazole contains a ketone group at position −5 of the benzimidazole ring which has implications on its metabolic pattern. For instance, carbonyl-reducing enzymes catalyse the NADPH-dependent conversion of FLBZ into its reduced metabolite (red-FLBZ) in sheep liver (Bártíková *et al.*, 2010). The aim of the current work was to assess the comparative FLBZ metabolism pattern in rat, swine and sheep liver cytosolic and microsomal fractions.

MATERIALS AND METHODS

Liver microsomal and cytosolic fractions were obtained from male ($n = 3$) and female ($n = 3$) Wistar rats, male Texel lambs ($n = 6$) and male Landrace piglets ($n = 4$). Flubendazole ketoreduction was assessed by the rate of red-FLBZ formation in the presence of NADPH. Incubations with red-FLBZ as substrate were also carried out in the presence of NADP+. Substrates were incubated at 100 μM (15 min at 37°C) and samples were analysed by HPLC. Statistical comparisons among species were performed using non-parametric ANOVA.

RESULTS

Both liver cytosolic and microsomal fractions from each animal species were able to metabolize FLBZ into red-FLBZ. The cytosolic production of the red-FLBZ was higher ($P < 0.05$) in sheep (1.86 ± 0.61 nmol min^{-1} mg^{-1}) compared to rats (0.13 ±

0.12 nmol min^{-1} mg^{-1}) and pigs (0.02 ± 0.00 nmol min^{-1} mg^{-1}). Male rat liver microsomes metabolized FLBZ into red-FLBZ to a greater extent ($P < 0.05$) than female (0.87 ± 0.25 versus 0.10 ± 0.06 nmol min^{-1} mg^{-1}). The production of red-FLBZ in liver microsomes was higher in sheep (1.62 ± 0.28 nmol min^{-1} mg^{-1}) compared to pigs (0.04 ± 0.02 nmol min^{-1} mg^{-1}, $P < 0.001$) and female rats (0.10 ± 0.06 nmol min^{-1} mg^{-1}, $P < 0.05$). A hydrolyzed metabolite (h-FLBZ) was only formed by both subcellular fractions from pig liver. The formation rate of h-FLBZ in swine hepatic microsomes was 7-fold higher ($P < 0.05$) compared to that observed for red-FLBZ. Only sheep liver cytosolic and microsomal fractions were able to produce the NADP+-dependent oxidation of red-FLBZ into FLBZ parent drug at 0.68 ± 0.07 and 0.64 ± 0.12 nmol min^{-1} mg^{-1}, respectively.

CONCLUSIONS

Phase I enzymes catalysing the reduction of xenobiotics containing a carbonyl group include short-chain dehydrogenases/reductases (SDR) (Cox Rosemond & Walsh, 2004). A wide array of xenobiotic substrates was found to be metabolized by cytosolic carbonyl reductases and the membrane-bound 11β-hydroxysteroid dehydrogenase, the most relevant enzymes within the SDR superfamily. The findings reported here demonstrate that FLBZ metabolic pattern may greatly differ among animal species. Considering the potential advantages that FLBZ may offer as an anthelmintic drug in both veterinary and human medicine, further work is required to understand the role of these carbonyl reducing enzymes on its metabolic fate across species.

REFERENCES

1. Bártícová, et al. (2010). Journal of Veterinary Pharmacology and Therapeutics, 32, 56–62.
2. Cox Rosemond & Walsh, (2004). *Drug Metabolism Reviews*, 36, 335–361.
3. Mackenzie & Geary (2011). *Expert Reviews Anti-infective Therapy*, 9, 497–501.
4. Maté *et al.*, (2008). *Biochemical Pharmacology*, 76, 773–783