JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. **39**, 95–97. doi: 10.1111/jvp.12248. SHORT COMMUNICATION

A functional model for feline P-glycoprotein

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van Beusekom, C. D., Lange, R., Schrickx, J. A. A functional model for feline P-glycoprotein. *J. vet. Pharmacol. Therap.* **39**, 95–97.

P-gp (ABCB1) belongs to the group of export transporters that is expressed in various species at biological barriers. Inhibition of P-gp can lead to changes in pharmacokinetics of drugs (drug–drug interactions), which can lead to toxicity and adverse side effects. This study aimed to establish a functional assay to measure the inhibitory potential of veterinary drugs on feline P-gp by means of fluorescence-associated flow cytometry of feline lymphoma cells. In this model, PSC833 and ivermectin potently inhibited P-gp function; cyclosporine and verapamil moderately inhibited P-gp function, whereas ketoconazole, itraconazole, diazepam, and its metabolites had no effect on P-gp function. This model can be used for testing the inhibitory potency of (new) drugs on feline P-gp.

(Paper received 1 October 2014; accepted for publication 15 June 2015)

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The multidrug resistance protein (MDR1/ABCB1) or permeability glycoprotein (P-gp) is a member of the ATP-binding cassette (ABC) transporters and can be found in, for example, liver, intestines, kidneys, and the brain, in many animal species (Mealey, 2004). Drugs that are substrates for P-gp are prevented from entering or excreted to the luminal side of these organs, and therefore, P-gp serves as a part of the biological barrier. Its role in pharmacokinetics is exemplified by a well-known veterinary clinical example of a dysfunction of P-gp in ivermectin-sensitive collie breeds (Mealey *et al.*, 2001) and by multidrug resistance in anticancer therapy (Darby *et al.*, 2011). Polymorphisms as well as drug–drug interactions at the level of P-gp are of clinical concern in both human and veterinary medicine (Schrickx & Fink-Gremmels, 2008; Zakeri-Milani & Valizadeh, 2014).

In cats, P-gp was first studied *in vitro* in feline lymphoma cells, where it was suggested that the basic structure of the feline ortholog and its role in multidrug resistance was essentially the same as in other species (Okai *et al.*, 2000). Organ distribution (Van der Heyden *et al.*, 2009) and P-gp expression in various tumors were later demonstrated in cats (Brenn *et al.*, 2008; Hifumi *et al.*, 2010; Van der Heyden *et al.*, 2011).

This study aimed to establish a functional assay to measure the inhibitory potential of veterinary drugs on feline P-gp, by means of fluorescence-associated flow cytometry of feline lymphoma cells. Drugs were selected based on P-gp inhibition in other animal species. The feline T-cell lymphoma cell lines were a generous gift from Y. Goto-Koshino (University of Tokyo, Japan). The FT-1 cell line (without expression of P-gp) was derived from an FeLV-positive thymic lymphoma of a cat, and the ADM-resistant subline FT-1/ADM (with confirmed P-gp expression) was selected from the FT-1 cells after several passages in a medium containing adriamycin (ADM) (Okai *et al.*, 2000). Cells were maintained in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal calf serum, penicillin (100 U/mL)/streptomycin (100 μg/mL), and 2 mM glutamine and subcultured every 3 days. Once every 3 passages, the FT-1/ADM cell line was cultured in medium containing 0.4 μg/mL doxorubicin. Expression of P-gp was confirmed by qPCR in the FT-1/ADM cell line using primers previously described by Okai *et al.* (2000), and at the same time, the absence of P-gp transcript was confirmed in the FT-1 cells (data not shown).

Initial experiments showed low uptake of rhodamine 123 (Rh123) in the FT-1/ADM cell line, due to the high efflux of the fluorescent dye. Therefore, instead of an efflux assay, an uptake assay was performed, as previously described for canine cells (Schrickx, 2014). In 96-well plates, a total number of 0.5×10^6 viable cells/well were incubated with 8 μM Rh123 and a concentration range of the selected drug simultaneously, for 30 min at 37 °C in an atmosphere of 5% CO_2. After washing with PBS, cell-associated fluorescence was measured with a flow cytometer (Accuri C6; BD Biosciences, San Jose, CA, USA). Differences in Rh123 fluorescence intensity between control cell samples (FL_c) and cell samples with the test compound (FL_s) were calculated according to equation 1. The background fluorescence intensity (FL_B) of cell samples that were not loaded with Rh123 was subtracted from each value.

$$\text{Relative uptake} = (FL_S - FL_B)/(FL_C - FL_B) \quad (1)$$

Concentration-dependent inhibitory effects were presented by sigmoidal curves, calculated in similar ways as Schrickx (2014). Incubation of the FT-1/ADM cells with 10 μM of the marker P-gp inhibitor PSC833 (Novartis Pharma) (Boesch *et al.*, 1991) was regarded as the maximum Rh123 efflux inhibition (I_{max} or 100% inhibition). The mean uptake of Rh123 by cells incubated in control medium (without PSC833 or any other of the tested compounds) was the minimum inhibition of Rh123 efflux (I_{min} or 0% inhibition). Inhibition curves with a variable slope were fitted for the concentration ranges, IC_{50} values were calculated using GraphPad Prism 6.01 software (San Diego, CA, USA), and values are expressed as mean ± SD of percentage uptake of at least three independent experiments.

Inhibition of Rh123 efflux was observed in the FT-1/ADM cell line and not in the FT-1 cell line, which confirmed the suitability of this model. The inhibitory potencies of the different drugs on Rh123 efflux in the FT-1/ADM cells are shown in Fig. 1. PSC833 and ivermectin were the most potent inhibitors, while cyclosporine inhibited Rh123 efflux to a lesser extent. Verapamil inhibited Rh123 efflux only for a small percentage at the highest soluble concentration (≤0.4% DMSO), whereas ketoconazole and itraconazole as well as diazepam and its metabolites had no influence on Rh123 efflux in the FT-1/ADM cells (data not shown).

IC_{50} values for PSC833 and ivermectin in the feline lymphoma cells (FT-1/ADM) were 2.09 ± 0.18 and 3.37 ± 0.16 μM, respectively. The IC_{50} value for cyclosporine could not be calculated accurately, as 100% inhibition could not be established by the tested concentration range. In general, IC_{50} values are higher in these feline lymphoma cells when compared to investigations performed on canine lymphoid cells (Schrickx, 2014; Zandvliet *et al.*, 2014), but the ranking order of inhibitory potency of the different compounds is comparable. However, a more in-depth interspecies comparison of P-gp characteristics is hampered by differences in the tested cell lines, originated from different tumorous cells.

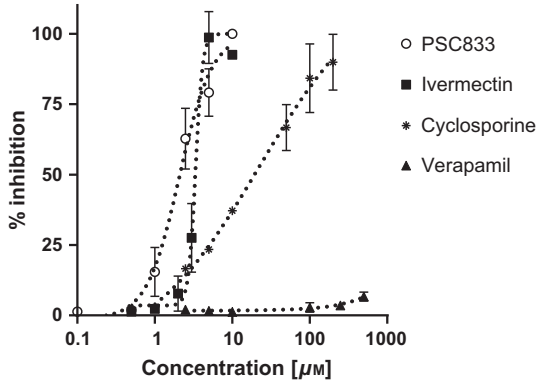

Fig. 1. Inhibitory effect of the marker P-gp inhibitor PSC833 and tested drugs (ivermectin, cyclosporine, and verapamil) on feline P-gp. Data represent the mean ± SD of at least three independent experiments with samples performed in duplicate.

In conclusion, the lymphoma cell assay used in this study can serve as an easy accessible *in vitro* model for testing the inhibitory potency of (new) drugs on feline P-gp.

ACKNOWLEDGMENTS

The authors thank Y. Goto-Koshino for the FT-1 and FT-1/ADM cell lines and M.A.M. Oosterveer-van der Doelen for her technical assistance.

CONFLICT OF INTEREST STATEMENT

None of the authors have to declare a conflict of interest. The study was sponsored by a PhD grant of Utrecht University.

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