Concomitant Simultaneous and Consecutive Treatment of Imidacloprid/Moxidectin Spot-on with Emodepside/Praziquantel Tablets in Adult Dogs

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ABSTRACT

Fourteen healthy adult Beagle dogs were included in this clinical study. Dogs randomly allocated to two study groups were treated on day 0 with a spot-on containing imidacloprid/moxidectin at the maximum approved dosage of 25 mg imidacloprid/kg bodyweight (bw) + 6.25 mg moxidectin/kg bw. Group 1 was treated at the same time orally with tablets containing emodepside/praziquantel at the maximum approved dosage of 1.9 mg emodepside/kg bw + 9.4 mg praziquantel/kg bw. Group 2 was treated on day 8 with emodepside/praziquantel tablets at the same dosage. These two different treatment scenarios were selected to compare a simultaneous treatment with a consecutive treatment. In the consecutive treatment an 8-day interval was chosen to ensure overlapping peak serum concentrations of emodepside and moxidectin. Dogs were fed directly after treatment. Profound safety assessments were performed pre- and post-treatment. Blood samples were drawn pre- and post-treatment to evaluate the hematology and clinical chemistry profile as well as the serum levels of moxidectin and emodepside. Neither compound-related adverse reactions nor treatment-related changes in the blood hematology or chemistry profile were seen. In this study reported here, it was clearly demonstrated that the concomitant treatment of both products applied either simultaneously or consecutively did not lead to any clinical nor laboratory abnormalities in adult Beagle dogs.

INTRODUCTION

A wide range of endo- and ectoparasites can concomitantly infect the dog. Although there are already several broad spectrum parasiticides for dogs on the market, none of them cover the whole endo- and/or ectoparasitic spectrum. Concomitant treatment with one or more products is therefore a common practice.

In the present study, two broad-spectrum antiparasitic products, emodepside/praziquantel tablets (Profender®) and imidacloprid/moxidectin spot-on (Advocate®), were administered to dogs either simultaneously or consecutively.

Profender® tablets for dogs, authorized in 2008 (Bayer Animal Health GmbH, Leverkusen, Germany), are a combination product containing emodepside and praziquantel. Emodepside is a semi-synthetic

derivate of PF1022A and belongs to the cyclic depsipeptides. Emodepside binds to a presynaptic latrophilin receptor which belongs to the group of G-protein coupled receptors.[33,14]. In addition, emodepside acts via a second target, a Ca++-activated K+ channel. This channel belongs to the large-conductance calcium- and voltage-activated potassium channels, termed SLO-1, which generally are important regulators of cell excitability. The end effect of emodepside is flaccid paralysis and death of the nematode.[16]

Praziquantel is an acylated pyrazino-isoquinoline derivative and has been used in veterinary medicine for many years as an effective active against cestodes and trematodes.[31,5]

Emodepside/praziquantel tablets are indicated for dogs suffering from, or at risk from, mixed parasitic infections caused by roundworms (immature and mature *Toxocara canis, Toxascaris leonina, Ancylostoma caninum, Uncinaria stenocephala, Trichuris vulpis*) and tapeworms (*Dipylidium caninum, Taenia* spp, mature and immature *Echinococcus multilocularis*, and *E. granulosus*).[3,26]

Advocate® spot-on for dogs (Bayer Animal Health GmbH, Leverkusen, Germany) is a solution for dermal application containing imidacloprid and moxidectin. Moxidectin is a macrocyclic lactone structurally within the milbemycin family and derived from the actinomycete *Streptomyces cyanogriseus spp Noncyanogenus*.[28] The primary mode of action results from moxidectin binding to glutamate-gated chloride channels of the parasites, leading to hyperpolarization of the neuronal cells that results in paralysis and death of the parasite.[10] Imidacloprid belongs to the group of neonicotinoide insecticides and acts as an agonist at nicotinic acetylcholine receptors (nAChRs) and shows selective toxicity for insects over vertebrates.[22] In addition to efficacy against gastrointestinal nematodes, imidacloprid/moxidection spot-on is efficacious for treatment and prevention of flea infestation (*Ctenocephalides felis*), treatment of ear mite infestation (*Otodectes cynotis*), prevention of heartworm disease (L3 and L4 larvae of *Dirofilaria immitis*) and *Angiostrongylus vasorum*, and treatment of sarcoptic mange (*Sarcoptes scabiei var. canis*) and demodicosis (*Demodex canis*).[15,6,20,11,19,12]

Emodepside/praziquantel tablets and imidacloprid/moxidectin spot-on were selected to assess the clinical impact of concomitant use, since both products may be administered simultaneously in a clinical setting.

Both emodepside and moxidectin are known to be substrates for P-glycoprotein (P-gp), the product of the multidrug resistance gene 1 (mdr1).[7,17] P-gp functions as a drug transport pump in various cell membranes including the blood brain barrier, transporting a variety of drugs from the brain back into the blood.[24]Although moxidectin is described as a weak P-gp substrate, co-administrations with other P-gp substrates could cause interactions at the level of the transporter. [13,21]

Potential clinical consequences of co-administration of emodepside and moxidectin have not previously been investigated. In the present study the two parasiticides, emodepside/praziquantel tablets and imidacloprid/moxidectin spot-on, were administered concomitantly to dogs simulating worst case scenarios: on the one hand, a simultaneous treatment, and on the other a consecutive treatment with overlapping maximum serum concentrations of emodepside and moxidectin. In addition, the maximum therapeutic dose of both compounds was applied. To test the worst case scenarios even further, animals in this study were fed immediately after treatment, which is in contrast to the label instructions to administer emodepside/praziquantel tablets only to fasted dogs and not to feed dogs until 4 hours post-treatment.

MATERIALS AND METHODS

Animals

Fourteen adult Beagle dogs (1.4 – 7.3 years, 3 male, 11 female) weighing between 8.4 and 14.7 kg were enrolled. The dogs were part of a regularly maintained re-

***Table 1**: Clinical observation and blood sampling time points*

Group 1 Simultaneous treatment	
Physical examination for study inclusion weighing, randomisation	Day -3
Clinical assessment	Day 0: pre-tr., hourly 1 to 6 h, 8 h Day 1 (24 h p.tr.)
Blood chemistry/haematology*	Day 0 pre-tr. Day 1 (24 h p.tr.) Day 2 (48 h p.tr.) Day 3 (72 h p.tr.)
Serum concentration* (moxidectin, emodepside, praziquantel)	Day 0: pre-tr., 1, 3, 5, 8 h Day 1 (24 h p.tr.) Day 3 (72 h p.tr.) Day 8
Group 2 Consecutive treatment	
Physical examination for study inclusion weighing, randomisation	Day -3
Clinical assessment	Day 0: pre-tr. Day 8: pre-tr., hourly 1 to 6 h, 8 h Day 9 (24 h p.tr.)
Blood chemistry/haematology*	Day 0 pre-tr. Day 8: pre-tr. Day 9 (24 h p.tr.) Day 10 (48 h p.tr.) Day 11 (72 h p.tr.)
Serum concentration* (moxidectin, emodepside, praziquantel)	Day 0: pre-tr. Day 3 (72 h p.tr.) Day 8 pre-tr., 1, 3, 5, 8 h Day 9 (24 h p.tr.) Day 11 (72 h p.tr.)

Pre-tr. = pre-treatment , p.tr. = post-treatment
**±12 minutes (mean: 3 minutes/group 1, 4 minutes/group 2)*

search colony and were returned to the colony after study completion. All animal procedures were approved by the responsible authorities and the animal welfare officer. Husbandry of animals complied with the European Commission guidelines for the accommodation of animals used for experimental and other scientific purposes (June 18, 2007/526/EC). The dogs were identified by ear tattoo number and each animal was housed in an individual pen during the study. They were fed once daily with a commercial dry dog food and water supply was provided ad libitum. All dogs were exposed to 12 h light and 12 h darkness. Temperature range was between 19° and 23°C and relative humidity between 33% and 50%.

Clinical observations

On day -3 a physical examination was performed with special emphasis on behavioural attitude, nutritional status, respiratory system, gastrointestinal system, and cardiovascular system. General health observations of the dogs were performed daily during the whole study period at cleaning and feeding activities. Clinical assessments

for signs of abnormal behaviour, salivation, vomiting, neurological signs, and tremor were performed pre- and post-treatment (see Table 1).

Treatment

The dogs were ranked by descending body weight, placed into blocks, and were allocated by random number draw within a block to two groups of seven dogs each. As pre-treatment clinical observations and baseline blood samples were performed, each dog acted as its own control. Especially for blood parameters with high inter-individual variations, the direct comparison of post-treatment to pre-treatment parameters was regarded as an accurate way to reflect the real impact on the individual dog.

All dogs received the maximum therapeutic dose, ie, the maximum theoretic dose a dog can receive if it is located at the lower margin of the body weight range. Day -3 body weights were used for both groups to calculate the dose on day 0, while day 7 body weights were used to calculate the dose on day 8 in group 2. Dogs of both study groups were treated on day 0 dermally with imidacloprid/moxidectin spot-on. The dosage applied was 25 mg imidacloprid/kg bw + 6.25 mg moxidectin/kg bw. This corresponded to a volume of 0.25 ml Advocate® for dogs/kg bw. Treatment was applied in accordance with the label instructions, ie, dermally at one spot between the shoulder blades.

Group 1 was treated at the same time orally with emodepside/praziquantel tablets at a dosage of 1.9 mg emodepside/kg bw

***Table 2**: Parameters tested for hematology and clinical chemistry*

Hematology	Clinical Chemistry
ADVIA® 120 Hematology System	IDEXX VetTest® 8008 Chemistry Analyzer
	Albumin (ALB) g/l
	Alkaline phosphatase (ALKP) U/l
	Alanine-aminotransferase (ALT) U/l
	Amylase (AMYL) U/l
	Aspartate-aminotransferase (AST) U/l
	Blood urea nitrogen (UREA) mmol/l
Red blood cell count $x10^6/\mu l$	Creatinine (CREA) µmol/l
Hemoglobin g/dl	Creatinkinase (CK) U/l
Hematocrit %	Cholesterol (CHOL) mmol/l
Mean corpuscular volume (MCV) fl	Gamma-glutamyl-transferase (γGT) U/l
White blood cell count $x10^3/\mu l$	Globulin (GLOB) g/l
White blood cell differential count $x10^3/\mu l$ / %	Glucose (GLU) mmol/l
Reticulocyte count $x10^6/\mu l$ / %	Lactate dehydrogenase (LDH) U/l
Platelet count	Lipase (LIPA) U/l
	Ammonium (NH3) µmol/l
	Total bilirubine (TBIL) µmol/l
	Total protein (TP) g/l
	Triglyceride (TRIG) mmol/l
	Calcium (Ca) mmol/l
	Magnesium (Mg) mmol/l
	Phosphorus (PHOS) mmol/l

Table 3: *Between-group comparison: Parameters showing both significant ($p < 0.05$) differences between group 1 and 2 and values outside the reference ranges*

	Time point p.tr. #	Statistical significance (group comparison)	Dogs outside reference range (high / low)	Comments to dogs outside reference range
Clinical chemistry				
CK	2 days p.tr.	Group 2 higher than group 1* (p = 0.0507)	1 dog in group 2: high	Elevation > 3x
LDH	3 days p.tr.	Group 1 higher than group 2 (p = 0.0398)	1 dog in group 1: high	Slight elevation

 # for group 2: after second treatment
**not significant, but approached significance (p = 0.0507)*
p.tr. = post-treatment

+ 9.4 mg praziquantel/kg bw. Group 2 was treated with the emodepside/praziquantel tablets on day 8 at the same dosage. The tablets were weighed and filed off with sandpaper to get the individual calculated tablet mass (tolerance + 1 mg). The dogs were observed at dosing and shortly after dosing to determine whether any tablet matter was regurgitated. All dogs were fed immediately after treatment on day 0 and 8.

Sampling

Blood samples were drawn for hematology, clinical chemistry, and for confirmation of serum levels of moxidectin and emodepside.

For the baseline blood was collected from all dogs on day 0 before treatment, enabling the dogs to act as their own control. For sampling time points see Table 1.

Blood samples were collected from the Vena jugularis. EDTA-K3 and lithium heparin containing blood collection systems were used as anticoagulants for hematological and serum chemistry analyses respectively, while plain serum collection systems were used for measurement of serum concentrations. EDTA containing blood samples were directly analyzed with an ADVIA® 120 Hematology System (Siemens Healthcare Diagnostics, Deerfield, IL, USA) in accordance with the manufacturer´s instructions. A total of 19 parameters were determined (see Table 2). Lithium heparin containing blood samples were centrifuged, plasma was harvested and directly analyzed with an IDEXX VetTest® 8008 Chemistry Analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA) in accordance with the manufacturer´s instructions. A total of 21 parameters were determined (see Table 2). The serum tubes were centrifuged, serum was harvested and frozen at -18°C until analysis.

Analysis of actives

For analysis, serum was deproteinized by mixing with acetonitrile and was subsequently centrifuged. The quantitative determination was performed by direct injection of an aliquot of the supernatant into a High Performance Liquid Chromatograph and detection by tandem mass spectrometry. The method was validated in the range from 2 to 1500 µg/l with mean recovery rates of 96 ± 9.6% for moxidectin and 105 ± 9.1% for emodepside.

Data were analyzed using non-compartmental pharmacokinetic methods with a commercial program (WinNonlin version 5.2, Pharsight® Corp., Mountain View, CA, USA).

Statistical analysis

Prior to analyses, all post-treatment clinical chemistry and hematology values were aligned between both treatment groups. Post-treatment values were compared to pre-treatment values by using the day 0 values

Table 4: *Pharmacokinetic parameters (geometric mean, CV%) of moxidectin and emodepside*

Parameter	Group	Moxidectin		Emodepside	
c_{max} (µg/l)	1	42.6	CV% = 67.3	140.3	CV% = 28.7
	2	51.8	CV% = 42.2	64.5	CV% = 243.1
t_{max} (h)	1	80.1 (= 3 days 7.2 h)	CV% = 117.8	1.5	CV% = 76.2
	2	152.6 (= 6 days 9.6 h)	CV% = 0.7	2.7	CV% = 0.7

c_{max} = peak serum concentration, t_{max} = time to peak serum concentration, CV% = coefficient of variation

for the baseline in both groups. Comparisons between the blood values of the two treatment groups were performed using group 1 values from days 1, 2, and 3, and group 2 values from days 9, 10, and 11.

Comparisons between groups utilized day 0 values as a baseline covariate, and a repeated measures analysis of covariance or a generalized linear mixed model analysis using binary data was used, depending upon the data distributions. In addition, separate analyses were performed within each treatment group, testing whether the pre-treatment baseline values of day 0 were significantly different from any of the three post-treatment values. All analyses used SAS version 9.3 software (SAS Institute Inc., Cary, NC, USA) with an alpha of 0.05 or less as statistically significant.

RESULTS

Clinical observations

All dogs tolerated the treatment well. One dog in group 2 regurgitated one tablet with a small amount of saliva between 7 to 16 minutes after application of the emodepside/praziquantel tablets (day 8) before feeding. The tablet that was found was immediately reapplied. The observed regurgitation is likely due to stress by restraining and deep tablet application into the mouth and therefore, not regarded as product related.

Blood hematology and chemistry

Only parameters that were outside the reference ranges given by the respective manufacturers of ADVIA® 120 Hematology System and IDEXX VetTest® 8008 Chemistry Analyzer, and that exhibited significant differences were considered relevant and are discussed here.

Statistical analyses were performed on all hematological and clinical chemistry parameters, specifically testing whether the pre-treatment baseline values were significantly different from any of the three post-treatment values for each separate treatment group. There were no significant changes observed in parameters outside the reference ranges.

Statistical analyses were also performed comparing the blood values between the two treatment groups. Results indicated significant changes in two parameters (CK, LDH) that were outside the reference ranges (see Table 3). In one dog in group 2, CK activity pre-treatment on day 0 was more than double the upper reference range (upper limit of reference range 200 U/l), but normal on day 8. On day 9 and 10, activity of CK increased again to more than 5-fold (1,059 and 1,907 U/l) with concurrent slight elevations of LDH. On day 11 CK activity had decreased to concentrations only slightly above the reference range and LDH activity was inside the reference range. As a consequence of this high CK activity of this single dog on day 10 the CK activity of group 2 nearly approached significantly higher levels on day 10 compared to group 1 ($p = 0.0507$). On day 9, another two dogs in group 2 had CK values that were more than double the upper reference range (881 and 453 U/l) with slightly increased LDH values, but these were normal on day 10.

Although on day 3 significantly higher activity of LDH in group 1 than in group 2 was observed ($p = 0.0398$), only one dog in group 1 had LDH activities slightly above

Figure 1: *Mean serum concentration time profile of moxidectin and emodepside for group 1*

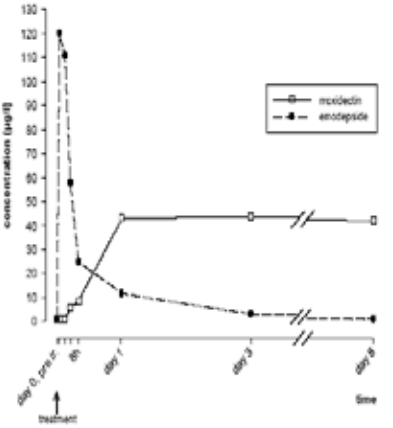
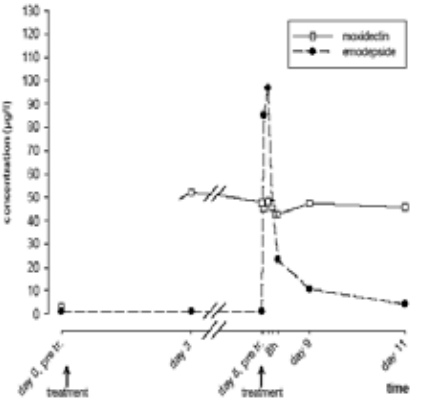


Figure 2: *Mean serum concentration time profile of moxidectin and emodepside for group 2*



the reference range on this day.

Serum concentration

Moxidectin concentrations were first detected 3 h after application and reached a plateau between days 1 and 11 (see Figures 1+2).

Emodepside concentrations were detected in serum at the first sampling time point of 1 h post-treatment and reached their peak at 1.5 h in group 1 and 2.7 h in group 2. Concentrations decreased thereafter with levels below the limit of quantification on day 8.

The calculated peak serum concentrations (c_{max}) and time to peak serum concentration (t_{max}) are summarized in Table 4.

DISCUSSION

The purpose of this study was to evaluate a concomitant treatment of imidacloprid/moxidectin (Advocate® spot-on) and emodepside/praziquantel (Profender® tablets). To simulate a treatment scenario that might have maximum impact, in addition to the common practice of simultaneous treatment, a consecutive treatment with an 8-day interval to ensure overlapping peak serum concentrations of emodepside and moxidectin was also investigated. This 8-day interval was chosen based on results of previous pharmacokinetic studies with imidacloprid/moxidectin spot-on.[30] In our study the time to peak serum concentrations of moxidectin was between 3 and 7 days post-treatment, but as moxidectin concentration remained from day 3 to day 11 on a plateau-like level, the overlapping of peak moxidectin and emodepside serum concentrations was achieved.

In previous studies, peak moxidectin concentrations of 15.3 µg/l and emodepside concentrations of 47 µg/l were found after application of 2.5 mg moxidectin/kg bw and 1.5 mg emodepside/kg bw (G. Beddies, unpublished data 2001, G. Ahr and O. Görgen, unpublished data 2007). In this study relative high mean peak serum concentrations of moxidectin (day 3-11: 42.6/51.8 µg/l) and emodepside (64.5/140.3 µg/l) were found, but a higher dosage of both actives was applied. These high serum levels confirmed high resorption and confirmed and met the demands of the worst case scenarios.

Label instructions for Profender® tablets are to treat only fasted dogs and to not feed earlier than 4 hours post-treatment, as neurological signs are more likely to occur in fed dogs.[8] Therefore, to simulate additional worst case scenarios that might have an impact, in this study all dogs were fed im-

mediately after treatment and the maximum therapeutic dose of the active ingredients of both products was applied. During clinical assessments no compound related adverse reactions were observed.

Concerning hematology and clinical chemistry, no changes in these profiles could be seen that were considered to be treatment related. Comparisons to baseline and between groups indicated that even when there were statistically significant changes, the values were nevertheless inside the reference ranges and therefore not clinically relevant. No relevant significant changes were seen in comparison to baseline.

In the between-group comparison two parameters (CK and LDH) were observed with statistical significant changes and values that were outside the reference range. Especially one dog in group 2 had more than 5-fold CK activity on day 9 and 10, while another two dogs in this group also had slight CK elevations on day 9. The cytosolic enzyme CK is a muscle specific enzyme with its main distribution in skeletal muscle and myocardium, while low concentrations are found in intestine and brain.[18,1] Although CK is considered a good and easily available indicator of muscle damage in dogs, false positives resulting from its high concentration in muscles and its possible leakage for non-specific reasons may occur.[2] Intramuscular injection itself has been shown to increase the enzyme levels[22] and even incorrect venipuncture can result in serum values elevated by at least 25% due to muscle puncture.[9] Hemolysis and hyperbilirubinaemia can also lead to falsely elevated CK values but have only slight effects if moderate.

Physical activity was described as the main factor for intra-individual variation, which occurred in this study during the frequent clinical assessments.[1] Another contributing factor might be accidental muscle puncture during blood sampling and hemolysis of the blood samples. As the ubiquitous LDH is found in high concentrations in muscle together with CK,[18] the concurrent slight elevations of LDH in these dogs seemed to be based on the same mechanisms.

All the results obtained in this study prove the safety of a concomitant simultaneous or consecutive treatment of imidacloprid/moxidectin spot-on (Advocate® spot-on) and emodepside/praziquantel tablets (Profender® tablets) in adult Beagle dogs. As moxidectin and emodepside are known to be substrates for P-glycoprotein (P-gp) and in this study no dogs with the mdr1 gene defect (mdr1-/-) were tested, the safety for this special case has still to be proven in further investigations.

CONCLUSION

None of the 14 dogs dosed with imidacloprid/moxidectin spot-on and emodepside/praziquantel tablets showed adverse events nor changes in their blood haematology or serum biochemistry profile during the course of the study that were considered to be treatment related. It was shown that concomitant treatment with both products applied either simultaneously or consecutively at the maximum therapeutic dose and with feeding directly after treatment was safe in adult Beagle dogs.

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