CHAPTER 6

Pharmacokinetic Modeling of the Disposition of Topical Ivermectin in Cattle as determined by Animal Behaviour

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Abstract

Animal behaviour is generally not recognised as a major determinant of drug disposition in animals. In the present study, pharmacokinetic modeling was applied to quantify the contribution of physiological cattle behaviour (licking) on the plasma and faecal disposition of topical ivermectin, a worldwide-used antiparasitic drug largely administered to cattle as a percutaneous formulation (pour-on). Six pairs of monozygotic twin cattle were given successively a single i.v. and two pour-on administrations of ivermectin at a 3-5-month interval. For the second pour-on administration, the twins were separated into two groups: a control group (lickers) and a group where self- and allo-licking were prevented. Ivermectin data obtained after i.v. and the second pour-on administrations in licking and non-licking twins were fitted simultaneously to a set of differential equations describing a seven-compartment model. Licking conferred a high intra- and inter-individual variability of systemic exposure after topical administration. We show that 58-87% of the pour-on dose was ingested, while only 10% of the dose was absorbed percutaneously. It results that 50-77% of the drug systemic absorption was achieved by the oral route. Approximately 72% of the ingested ivermectin transited directly into faeces, increasing by 5-fold the environmental burden of parent drug. This is of ecological interest, given the potential of ivermectin to adversely affect dung-degrading insects. We conclude that topical administration does not guarantee a controlled drug delivery in cattle. More importantly, simulations revealed that non-treated cattle could get easily contaminated by allo-licking, raising the public health problem of unexpected drug residues in edible tissues.
Introduction

Licking is an important part of the natural grooming behaviour of animals in many species. In cattle, it serves an important physiological function in skin and hair hygiene, and plays a major role in the establishment and maintenance of the herd social structure (Simonsen, 1979; Sato et al., 1991, 1993; Krohn, 1994). However, despite the increasing use of percutaneous drug formulations in both companion and food-producing animals (see Magnusson et al., 2001), the influence of licking on the disposition of topical drugs has never been documented. Over the last twenty years, the percutaneous route has received considerable interest in veterinary medicine for local and/or systemic delivery of drugs (Hennessy, 1997; Magnusson et al., 2001; Riviere and Papich, 2001). This application technique is particularly convenient for the owner who can easily apply the treatment himself with minimal risk of injury and minimal animal distress (Hennessy, 1997; Rothen-Weinhold et al., 2000). Furthermore, topical administration avoids the problems of hepatic first pass metabolism and of drug degradation in the gastrointestinal tract following oral administration, and therefore may be a good alternative to the oral route to achieve systemic therapeutic effects (Hennessy, 1997; Magnusson et al., 2001).

Ivermectin is a worldwide-used antiparasitic drug, which is routinely administered to millions of cattle per year for systemic effects. As for other endectocides (doramectin, moxidectin, eprinomectin), the topical "pour-on" formulation of ivermectin has largely displaced the conventional injectable formulation in farming practice. In a recent study (Laffont et al., 2001), we showed that the disposition of pour-on ivermectin in cattle was markedly modified with restriction of animal licking. Prevention of licking resulted in a significantly lower absolute bioavailability of ivermectin, in a two-times longer plasma elimination half-life, and overall in a ten-times lower elimination of the parent drug in faeces (Laffont et al., 2001). Altogether, these results suggested that under normal licking conditions, a large amount of the topical drug reached the systemic circulation by oral rather than percutaneous absorption. The raw data of this study on ivermectin in non-licking and control cattle are used in the present paper for the purpose of pharmacokinetic modeling. The aim was to quantify the actual contribution of licking to the systemic availability of pour-on ivermectin and to the excretion of the parent drug in faeces. The extent of ivermectin faecal excretion is indeed of special concern, given the potential negative environmental impact caused by the effect of the parent drug against some beneficial dung-breeding and dung-degrading insects (for review, see McKellar, 1997). We also present original data
on another pour-on administration of ivermectin performed in the same animals, in order to assess the intra-individual variability of exposure of the treated cattle.

This paper addresses the general problem of controlled drug delivery by topical application in animals, and discusses the consequences of animal social licking in terms of public health and food safety.

Materials and Methods

Study design and animals

The material and methods have been extensively described in the original paper (Laffont et al., 2001), except for information related to the first pour-on administration (PO1). Briefly, six pairs of monozygotic twin Holstein cattle (467 ± 19 kg b.wt., 3 years old) obtained by micromanipulation (Ozil et al., 1982) were used in the experiments. Each cattle received a single i.v. administration of injectable ivermectin (IVOMEC® injectable, Merial, Lyon, France; 200 µg/kg b.wt.) and two topical administrations of ivermectin (IVOMEC® pour-on, Merial, Lyon, France) at the standard dose of 500 µg/kg b.wt. All administrations were performed at a 3- to 5-month interval. For the second pour-on administration (PO2), the twins were separated into two groups of six animals. In the control group (the lickers), animals were kept in individual tie-stalls, each cattle being tethered with a loose chain so it could lick itself and its immediate neighbours. In the other group (the non-lickers), each animal was isolated from the others by a screen and was fitted with a wooden neck collar to prevent self-licking. Blood was collected regularly at 17 times up to 28 days after application of PO1, at 22 times up to 44 days after application of PO2, and at 20 times up to 31 days following i.v. administration. Total faeces were collected over 24 hours on days 4 and 14 after application of PO1. Following application of PO2, total faeces were collected for a period of 6 h (from 09:00 to 15:00 h) on days 1, 2, 3, 4, 7, 14, 18, 22 and 28 post-administration.

Analytical method

Concentrations of ivermectin (22,23-dihydroavermectin B1a) in plasma and in wet faeces were measured using a high-performance liquid chromatography (HPLC) technique (Alvinerie et al., 1987). The lower limit of quantification for ivermectin was 0.05 ng/mL for the plasma and 0.5 ng per g of wet faeces for the faecal samples. The limit of detection for ivermectin in plasma was 0.01 ng/mL. Accuracy and precision (intra-assay variation) expressed as relative standard
deviation were less than 8 and 6%, respectively.

**Pharmacokinetic analysis**

**Non-compartmental approach.** Individual areas under the plasma concentration-time curve, AUC(0-infinite), were computed using the trapezoidal rule. The extrapolated area (from the last sample to infinity) was calculated by dividing the last plasma concentration measured by the slope of the terminal phase (estimated in a semi-logarithmic scale).

Ivermectin (total) plasma clearance (CL\textsubscript{tot}) was calculated as the ratio of the administered dose (200 µg/kg) divided by the AUC (0-infinite) obtained for the i.v. route:

\[
\text{CL}_{\text{tot}} = \text{Dose}_{\text{i.v.}} / \text{AUC}_{\text{i.v.}} \quad \text{Eq. 1}
\]

The systemic availability for topical ivermectin (F\textsubscript{tot}) was calculated using the ratio of the AUCs (0-infinite) obtained after topical (AUC \textsubscript{pour-on}) and i.v. administrations (AUC \textsubscript{i,v.}) corrected by the ratio of the administered doses:

\[
F_{\text{tot}} (\%) = (\text{AUC}_{\text{pour-on}} / \text{AUC}_{\text{i,v.}}) \times (\text{Dose}_{\text{i,v.}} / \text{Dose}_{\text{pour-on}}) \times 100 \quad \text{Eq. 2}
\]

Cumulative amounts of parent ivermectin eliminated in faeces were calculated by integration of the faecal excretion rate profile as a function of time, using the trapezoidal rule. The faecal excretion rates were obtained as the total amount of parent drug eliminated in faeces within the collection interval (i.e., the product of the faecal concentration of ivermectin and the weight of wet faeces), divided by the time of collection.

**Data modeling.** Twin cattle were considered as the same animal taken under normal and restricted licking conditions. Each pair of twins was fitted separately. The pharmacokinetic model presented in Fig. 1 was selected among other different models to fit simultaneously the plasma and faecal ivermectin data obtained in the licking and non-licking twins after pour-on administration (PO2) and the i.v. plasma ivermectin data of the two twin cattle. The model was assumed dose-independent, but was not time-independent since the rate constant of ingestion of topical ivermectin (Ka) was described as a biexponential function of time. It was defined by the following equations:
Figure 1. Disposition model for ivermectin after i.v. and topical (pour-on) administration in one pair of licking and non-licking monozygotic twin cattle. $K_{ij}$ is the first-order rate constant of transfer from compartment $i$ to compartment $j$. $K_{10}$ is the first-order rate constant of elimination from compartment 1. The rate constant $K_a$ traduces the ingestion of topical ivermectin by licking and is modelised as a biexponential function of time. The i.v. dose (200 µg/kg) is introduced in compartment 1 (plasma), while the pour-on dose (500 µg/kg) is introduced in compartment 5 (at the surface of the skin). The subscripts "L" and "nL" refer to the "licking" (control) or "non-licking" twin, respectively. "GIT 1", gastrointestinal compartment 1. "GIT 2", gastrointestinal compartment 2. "PT", peripheral tissues.
Pharmacokinetic modeling of the disposition of topical ivermectin in cattle

\[ Cp = \frac{X_1}{V_1} \quad \text{Eq. 3} \]

\[ \frac{dX_1}{dt} = K_{21} \times X_2 + K_{31} \times X_3 + K_{51} \times X_5 + K_{61} \times X_6 - (K_{10} + K_{12} + K_{13}) \times X_1 \quad \text{Eq. 4} \]

\[ \frac{dX_2}{dt} = K_{12} \times X_1 - K_{21} \times X_2 \quad \text{Eq. 5} \]

\[ \frac{dX_3}{dt} = K_{13} \times X_1 - (K_{31} + K_{34}) \times X_3 \quad \text{Eq. 6} \]

\[ \frac{dX_4}{dt} = K_{34} \times X_3 + K_{64} \times X_6 \quad \text{Eq. 7} \]

\[ \frac{dX_5}{dt} = - (K_{51} + K_{a(t)} + K_{57}) \times X_5 \quad \text{Eq. 8} \]

\[ \frac{dX_6}{dt} = K_a (t) \times X_5 - (K_{61} + K_{64}) \times X_6 \quad \text{Eq. 9} \]

\[ \frac{dX_7}{dt} = K_{57} \times X_5 \quad \text{Eq. 10} \]

where \( Cp \) is the concentration of ivermectin in plasma (compartment 1, volume \( V_1 \)), \( X_i \) is the amount of ivermectin in compartment \( i \), \( K_{ij} \) is the first-order rate constant of transfer from compartment \( i \) to compartment \( j \), and \( K_{10} \) is the first-order rate constant of elimination from compartment 1.

For the disposition of topical ivermectin in non-lickers, \( K_a \) (the constant of transfer from compartment 5 to compartment 6) was set to zero. For the disposition of topical ivermectin in control animals (the lickers), \( K_a \) was empirically described as a function of time (Eq. 11), increasing up to a maximum and then decreasing (see discussion).

\[ K_a (t) = A \times \left[ \exp (-B \times t) - \exp (-C \times t) \right] \quad \text{Eq. 11} \]

where the parameters \( A, B \) and \( C \) are expressed in \( \text{h}^{-1} \).

The i.v. dose (200 \( \mu \text{g/kg} \)) was introduced into the plasma compartment, while the pour-on dose (500 \( \mu \text{g/kg} \)) was introduced into compartment 5 representing the skin surface. \( K_{12}, K_{21}, K_{13}, K_{31} \) are the distribution rate constants between the plasma and the peripheral compartments 2 and 3. The rate constant \( K_{10} \) relates to the elimination of ivermectin from plasma by metabolism, while the rate constant \( K_{34} \) traduces the removal of parent ivermectin from the gastrointestinal tract (GIT 2;
compartment 3) into faeces (compartment 4). $K_{51}$ is the rate constant for the systemic absorption of topical ivermectin via the skin. It was assumed that $K_{51}$ did not differ between lickers and non-lickers (see discussion). The rate constant $K_{57nL}$ accounts for the fraction of topical ivermectin which was not systemically available by the percutaneous route in the non-licking cattle (e.g. left at the skin surface, photo-degraded, or metabolised by the enzymes of the skin). In control animals (the lickers), the topical drug could be absorbed through the skin ($K_{51}$) or ingested by licking ($K_a$). One fraction of the ingested ivermectin was then absorbed in plasma ($K_{61}$), while the remaining fraction transited unchanged into faeces ($K_{64}$). The model assumes that the local disposition of ivermectin is not the same at all sites of the digestive tract (see discussion). The rates of drug absorption and elimination were indeed different in the gastrointestinal compartments 3 and 6 (GIT 1 and GIT 2, respectively). In lickers, the rate constant $K_{57L}$ refers to the fraction of topical ivermectin which was neither ingested nor systemically-available by the percutaneous route. First pass metabolism of ivermectin by the oral route was considered negligible, given the low value of the drug total clearance (overall coefficient of extraction < 1% in cattle). $T_{\text{lag-L}}$ is the delay to oral absorption, and $T_{\text{lag-nL}}$ is the delay to percutaneous absorption.

The differential equations were solved numerically using the SCIENTIST® program (MicroMath® Scientific Software, Inc., Version 2.01). The goodness of fit was assessed by examination of the lines of best fit and of residual patterns, taking into account a modified Akaike information criterion. The data points were weighed by the inverse of the squared observed values ($1/Y^2$) for ivermectin plasma concentrations, and by the inverse of the observed values ($1/Y$) for the amounts of ivermectin excreted in faeces. The fitting was performed stepwise: 1) the i.v. plasma concentrations of ivermectin obtained in the two twins were fitted simultaneously to equations 3-6, 2) the i.v. plasma data of the twins and the pour-on (plasma and faecal) data of the non-licker were fitted simultaneously using equations 3-10 (the estimates yielded by the first fitting were used as initial values for the parameters), and 3) the pour-on (plasma and faecal) data of the licking and non-licking twins and the i.v. plasma data of the two cattle were fitted simultaneously using the equations 3-11. The estimates yielded by the second fitting were taken as initial values for the parameters.

Pharmacokinetic parameters were calculated from the final estimates of the model parameters. Ivermectin total (plasma) clearance, $CL_{\text{tot IVM}}$, was calculated for each pair of twins according to the following equation (for demonstration, see
Nakashima and Benet, 1988):

\[ \text{CL}_{\text{tot IVM}} = V1 \times \left( K10 + K34 \times \frac{K13}{K31+K34} \right) \]  

Eq. 12

The relative importance of the oral and percutaneous routes in the removal of ivermectin from the skin was assessed for each licking cattle by simulation of X1, X6 and X7 using equations 4 and 8-10 with K10, K12, K13, K61, K64 equal to zero. Simulations were performed over a period of 6000 h, until no ivermectin was left in compartment 5. The fraction of topical ivermectin absorbed though the skin, ingested (f_{ingested}), and remaining (left on the skin or degraded) were calculated as the final amount of ivermectin obtained by simulation in compartment 1, 6 and 7, respectively, divided by the applied dose of pour-on (500 µg/kg).

The absolute oral bioavailability of ivermectin (F_{oral}) was estimated by modeling:

\[ F_{\text{oral}} (%) = \left( \frac{K61}{K61 + K64} \right) \times 100 \]  

Eq. 13

The percentage of topical ivermectin systemically-available by the oral route was obtained as \( f_{\text{ingested}} \times F_{\text{oral}} (%) \).

**Simulation of the ingestion of topical ivermectin with increased rates of percutaneous absorption**

The influence of the rate of percutaneous absorption on the extent of drug ingestion was examined. Cumulative amounts of ivermectin ingested and absorbed through the skin after topical application were simulated for various magnitudes of percutaneous absorption rates. Simulations were performed using the estimated parameters obtained in each pair of twins (see Table 2). Equations 4 and 8-10 were used for the simulations with K10, K12, K13, K61, K64 equal to zero.

**Simulation of the exposure of non-treated animals following cross-contamination by allo-licking**

The possibility of the cross-contamination of non-treated cattle by licking of those topically-treated was examined. Simulations were carried out to evaluate the minimal amount of ivermectin that had to be licked to achieve non-negligible plasma exposure of untreated cattle. Residues of ivermectin in milk were given as an indicator of the contamination of food. The simulations were performed for each pair of twins using the final estimated parameters (listed in Table 2). For simulation of a single oral uptake, the dose was introduced into compartment 6. In the case of
the ingestion of topical ivermectin by multiple oral uptake (modelised by Ka), the
dose was introduced into compartment 5 with $K_{51}$ and $K_{57L}$ equal to zero, and
equations 3-9 and 11 were used for the simulations. Residues of ivermectin in milk
were predicted from the corresponding plasma concentrations of ivermectin, given
a milk/plasma concentration ratio of 0.766 (Toutain et al., 1988).

Statistical analysis
Statistical analysis was performed using the SYSTAT® 8.0 (SPSS Inc., Chicago,
IL) software. ANOVA was used to compare the within-pair and between-pair
variability of plasma exposure (AUC) and of ivermectin total clearance after i.v.
administration in the 6 pairs of twins. ANOVA was also applied to compare the
intra- and inter-individual variability of plasma exposure (AUC) in the licker group
after pour-on administration. A $p < 0.05$ was considered as significant. The results
were expressed as means ± SD.

Results

Intravenous administration of ivermectin
The plasma concentration profiles of ivermectin obtained in the twelve cattle
were very homogenous (Fig. 2A). It must be noted that for all pairs of twins, the
plasma concentration-time curves of the two twins were exactly superposed (see
Fig. 3 for a representative pair of twins). Furthermore, the variability in plasma
AUC was significantly lower between the twins (coefficient of variation (CV) of
5%) than that between the different pairs of twins (CV of 25%) ($p < 0.01$). Similar
results were obtained for ivermectin total clearance, with a lower within-pair than
between-pair variability ($p < 0.01$). Altogether, these results consolidate the choice
of using monozygotic twins for the purpose of modeling.

The plasma data were well fitted by the model, as it is shown for a
representative pair of twin cattle in Fig 3. The individual total clearances of
ivermectin calculated by non-compartmental analysis were consistent with those
estimated by modeling (Table 1; see the original article for other pharmacokinetic
parameters: Laffont et al., 2001).

Pour-on administration of ivermectin
The plasma concentration-time profiles of ivermectin obtained after application
of PO2 are presented in Figure 2B. By comparison with the i.v. route, the systemic
exposure of animals was highly variable, depending on their ability to lick or not.
Figure 2. Ivermectin plasma concentration-time curves in 6 pairs of monozygotic twin cattle after i.v. administration (Fig. 2A) and topical (pour-on) administration (Fig. 2B). The twins were separated in two groups of 6 animals: the licker group (filled symbols) and the non-licker group (open symbols). For the pour-on administration, self- and allo-licking were prevented in the cattle of the non-licker group.
Figure 3A shows different slopes for the plasma terminal phase between i.v. and pour-on administrations in non-lickers. This “flip-flop” phenomenon (extensively described in the original article: Laffont et al., 2001) does not occur under normal licking conditions and shows that, in contrast to oral absorption, the percutaneous absorption of ivermectin is a very slow process limiting the drug plasma elimination. Cumulative amounts of parent ivermectin excreted in faeces under normal or restricted liking conditions are presented in Fig. 4A. They show major differences in the faecal excretion of the parent drug between licking and non-licking animals (see also Table 1).
**Figure 4.** Faecal excretion of parent ivermectin (IVM) following topical application of 6 pairs of monozygotic twin cattle (PO2). Twins were separated into two groups of 6 animals: the non-licker group (open circles), in which self-and allo-licking were prevented, and the licker group (filled circles, control group). A) Cumulative amounts of ivermectin excreted in faeces. B) Ivermectin faecal excretion rates. Open triangles and open squares refer to a previous pour-on administration (PO1) in the same animals (licker and non-licker groups, respectively), with this time no restriction of licking in any of the two groups.

**Figure 3.** Experimental (symbols) and simulated (line) ivermectin data after i.v. and topical (pour-on) administration in a representative pair of monozygotic twin cattle. Circle symbols, plasma concentrations of ivermectin after i.v. administration. Triangle symbols, cumulative amounts of parent ivermectin excreted in faeces after topical administration. Square symbols, plasma concentrations of ivermectin after topical administration. Licking was prevented in one of the two twins (open symbols; non-licking cattle), the other twin serving as a control (filled symbols; licking cattle). A) semi-logarithmic scale. B) arithmetic scale.
For the cattle of the non-licker group, it was possible to compare the disposition of pour-on ivermectin in the same animal under normal (PO1) and restricted (PO2) licking conditions. As shown in Fig. 4B, the faecal excretion rates observed in these cattle after application of PO1 match those found in their twins (licker group) following administration of PO1 and PO2. The overall bioavailability of pour-on ivermectin was not significantly increased with licking (24 ± 6.4 % in PO1 vs 22 ± 6.6 % in PO2), but more importantly no flip-flop phenomenon was observed.

Taken under normal licking conditions, all cattle showed a high variability of plasma exposure. After application of PO1, the difference in ivermectin bioavailability within the same pair of twins could be as high as 70%. In the licker group, the plasma AUC of ivermectin in a same animal was multiplied by a factor of 0.6 to 2.3 from one pour-on application to the other. The intra-individual variability (CV of 41%) was not significantly lower than the inter-individual variability (CV of 55%).

The plasma and faecal data obtained for ivermectin after pour-on administration were well fitted by the model for all pairs of twins (Table 1; see Fig. 3 for a representative pair of twins). In one pair of twins however, the predicted faecal data underestimated by 35% the observed amounts of parent drug recovered in the faeces of the licker, which will be further discussed. The estimated parameters of the overall disposition model presented in Fig. 1 are listed in Table 2. It is noteworthy that the parameters showing the highest coefficients of variation were those associated with the ingestion of the topical drug by licking (A, B, C, K61, K64, K57, T lag-L). Following topical administration, pharmacokinetic modeling gave a mean absolute bioavailability of ivermectin of 32 ± 13.8% in lickers and of 23 ± 7.5% in non-lickers (see Table 3), which is very close to the values obtained by non-compartmental analysis (33 ± 18.6% and 22 ± 6.6%, respectively).

The model indicates that 58-87% of the topically-applied ivermectin was actually ingested by licking (Table 3), and that 50-77% of the drug systemic absorption was achieved by the oral route (corresponding to 22% of the applied dose; Table 3) vs 23-50% by the percutaneous route (10% of the applied dose; Table 3). Oral absolute bioavailability of ivermectin was estimated by modeling (Eq. 8), and tended to be higher than for the percutaneous route (28 ± 13.2% vs 22 ± 6.6%, respectively). Together with the large ingestion of drug, this explains the higher overall bioavailability of pour-on ivermectin in lickers (Ftot of 32 ± 13.8%), compared to non-licking cattle (Ftot of 23 ± 7.5%) (Table 3).
Table 1. Ivermectin total clearance (CL<sub>total</sub>) in cattle, and evaluation of the contribution of licking to the faecal excretion of parent ivermectin after topical application to cattle.

<table>
<thead>
<tr>
<th>Pair number</th>
<th>CL&lt;sub&gt;total&lt;/sub&gt; (mL/day/kg)</th>
<th>% dose eliminated unchanged in the faeces of lickers</th>
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<tbody>
<tr>
<td></td>
<td>non-comp.</td>
<td>comp.</td>
</tr>
<tr>
<td></td>
<td>(0-28 days)</td>
<td>(0-28 days)</td>
</tr>
<tr>
<td>Non-lickers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>230.8</td>
<td>227.9</td>
</tr>
<tr>
<td>2</td>
<td>286.0</td>
<td>322.9</td>
</tr>
<tr>
<td>3</td>
<td>332.7</td>
<td>325.1</td>
</tr>
<tr>
<td>4</td>
<td>283.6</td>
<td>296.3</td>
</tr>
<tr>
<td>5</td>
<td>236.7</td>
<td>258.2</td>
</tr>
<tr>
<td>6</td>
<td>225.6</td>
<td>233.0</td>
</tr>
<tr>
<td>mean ± SD</td>
<td>266 ± 42.2</td>
<td>277 ± 43.6</td>
</tr>
<tr>
<td>Lickers</td>
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<tr>
<td>1</td>
<td>211.1</td>
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</tr>
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<td>6</td>
<td>210.2</td>
<td>233.0</td>
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<tr>
<td>mean ± SD</td>
<td>274 ± 68.5</td>
<td>277 ± 43.6</td>
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Compartmental (comp.) and non-compartmental (non-comp.) analysis were performed in the 6 pairs of monozygotic twin cattle.
<table>
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<th>Parameters</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>mean</th>
<th>%CV</th>
</tr>
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<td>$V_1$ (L/kg)</td>
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<td>0.084</td>
<td>0.080</td>
<td>0.093</td>
<td>0.074</td>
<td>0.058</td>
<td>0.078</td>
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<td>$K_{10}$ (h$^{-1}$)</td>
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<td>0.101</td>
<td>0.105</td>
<td>0.077</td>
<td>0.094</td>
<td>0.088</td>
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<tr>
<td>$K_{12}$ (h$^{-1}$)</td>
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<td>0.283</td>
<td>0.358</td>
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<tr>
<td>$K_{21}$ (h$^{-1}$)</td>
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<td>0.016</td>
<td>0.019</td>
<td>0.020</td>
<td>0.017</td>
<td>0.021</td>
<td>0.018</td>
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<tr>
<td>$K_{64}$ (h$^{-1}$)</td>
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<td>$K_{61}$ (h$^{-1}$)</td>
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<td>0.008</td>
<td>1.868</td>
<td>0.044</td>
<td>0.011</td>
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<tr>
<td>$K_{57_L}$ (h$^{-1}$)</td>
<td>0.00011</td>
<td>0.00058</td>
<td>0.00181</td>
<td>0.00000</td>
<td>0.00071</td>
<td>0.00223</td>
<td>0.00091</td>
<td>101</td>
</tr>
<tr>
<td>$K_{57_nL}$ (h$^{-1}$)</td>
<td>0.00269</td>
<td>0.00192</td>
<td>0.00245</td>
<td>0.00176</td>
<td>0.00143</td>
<td>0.00216</td>
<td>0.00207</td>
<td>22</td>
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<tr>
<td>$K_{13}$ (h$^{-1}$)</td>
<td>1.760</td>
<td>3.872</td>
<td>3.693</td>
<td>2.096</td>
<td>1.673</td>
<td>3.242</td>
<td>2.723</td>
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<tr>
<td>$K_{31}$ (h$^{-1}$)</td>
<td>0.642</td>
<td>0.883</td>
<td>0.918</td>
<td>0.484</td>
<td>0.491</td>
<td>0.889</td>
<td>0.718</td>
<td>28</td>
</tr>
<tr>
<td>$K_{34}$ (h$^{-1}$)</td>
<td>0.026</td>
<td>0.015</td>
<td>0.018</td>
<td>0.006</td>
<td>0.021</td>
<td>0.021</td>
<td>0.018</td>
<td>37</td>
</tr>
<tr>
<td>$TLAG_L$ (h)</td>
<td>37.1</td>
<td>14.3</td>
<td>27.4</td>
<td>20.0</td>
<td>0.0</td>
<td>5.4</td>
<td>17.4</td>
<td>79</td>
</tr>
<tr>
<td>$TLAG_nL$ (h)</td>
<td>6.8</td>
<td>5.4</td>
<td>5.0</td>
<td>2.0</td>
<td>7.1</td>
<td>5.7</td>
<td>5.3</td>
<td>35</td>
</tr>
<tr>
<td>$A$ (h$^{-1}$)</td>
<td>0.0186</td>
<td>0.0480</td>
<td>0.0042</td>
<td>0.0093</td>
<td>0.0108</td>
<td>0.0114</td>
<td>0.0170</td>
<td>93</td>
</tr>
<tr>
<td>$B$ (h$^{-1}$)</td>
<td>0.00325</td>
<td>0.00215</td>
<td>0.00096</td>
<td>0.00179</td>
<td>0.00158</td>
<td>0.00241</td>
<td>0.00202</td>
<td>39</td>
</tr>
<tr>
<td>$C$ (h$^{-1}$)</td>
<td>0.00731</td>
<td>0.00308</td>
<td>0.04622</td>
<td>0.02854</td>
<td>0.01658</td>
<td>0.02418</td>
<td>0.02098</td>
<td>75</td>
</tr>
<tr>
<td>$K_{51}$ (h$^{-1}$)</td>
<td>0.00052</td>
<td>0.00037</td>
<td>0.00052</td>
<td>0.00079</td>
<td>0.00070</td>
<td>0.00065</td>
<td>0.00059</td>
<td>25</td>
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</tbody>
</table>

The plasma (i.v., pour-on) and faecal (pour-on) ivermectin data of six pairs of monozygotic twin cattle were used for the compartmental analysis. Twins were separated as "lickers" and "non-lickers": licking was prevented in one twin, the other twin serving as a control. Each pair of twins was fitted separately. "L", Lickers; "nL", Non-lickers.
Table 3. Absolute bioavailability ($F_{\text{total}}$) of pour-on ivermectin in cattle, and evaluation of the extent of ingestion, oral absorption, and percutaneous absorption of ivermectin after topical application.

<table>
<thead>
<tr>
<th>Pair number</th>
<th>non-comp. analysis</th>
<th>comp. analysis</th>
<th>ingested</th>
<th>absorbed orally</th>
<th>absorbed through the skin</th>
<th>left</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-lickers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17.4</td>
<td>16.2</td>
<td>—</td>
<td>—</td>
<td>16.2</td>
<td>83.8</td>
</tr>
<tr>
<td>2</td>
<td>15.6</td>
<td>16.2</td>
<td>—</td>
<td>—</td>
<td>16.2</td>
<td>83.8</td>
</tr>
<tr>
<td>3</td>
<td>16.9</td>
<td>17.5</td>
<td>—</td>
<td>—</td>
<td>17.5</td>
<td>82.5</td>
</tr>
<tr>
<td>4</td>
<td>30.1</td>
<td>31.0</td>
<td>—</td>
<td>—</td>
<td>31.0</td>
<td>69.0</td>
</tr>
<tr>
<td>5</td>
<td>29.9</td>
<td>32.8</td>
<td>—</td>
<td>—</td>
<td>32.8</td>
<td>67.2</td>
</tr>
<tr>
<td>6</td>
<td>24.2</td>
<td>23.2</td>
<td>—</td>
<td>—</td>
<td>23.2</td>
<td>76.8</td>
</tr>
<tr>
<td><strong>mean ± SD</strong></td>
<td>22 ± 6.6</td>
<td>23 ± 7.5</td>
<td>—</td>
<td>—</td>
<td>23 ± 7.5</td>
<td>77 ± 7.5</td>
</tr>
<tr>
<td><strong>Lickers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35.6</td>
<td>37.8</td>
<td>83.4</td>
<td>24.0</td>
<td>13.8</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>18.2</td>
<td>18.0</td>
<td>80.5</td>
<td>10.1</td>
<td>7.9</td>
<td>11.7</td>
</tr>
<tr>
<td>3</td>
<td>21.4</td>
<td>20.4</td>
<td>58.4</td>
<td>10.2</td>
<td>10.2</td>
<td>31.4</td>
</tr>
<tr>
<td>4</td>
<td>68.4</td>
<td>56.0</td>
<td>86.6</td>
<td>42.6</td>
<td>13.4</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>22.9</td>
<td>28.4</td>
<td>80.1</td>
<td>18.7</td>
<td>9.7</td>
<td>10.3</td>
</tr>
<tr>
<td>6</td>
<td>29.6</td>
<td>31.1</td>
<td>67.8</td>
<td>23.9</td>
<td>7.3</td>
<td>25.0</td>
</tr>
<tr>
<td><strong>mean ± SD</strong></td>
<td>33 ± 18.6</td>
<td>32 ± 13.8</td>
<td>76 ± 10.8</td>
<td>22 ± 12.0</td>
<td>10 ± 2.7</td>
<td>13 ± 12.4</td>
</tr>
</tbody>
</table>

Compartmental (comp.) and non-compartmental (non-comp.) analysis were performed in the 6 pairs of monozygotic twin cattle.
Approximately 72% of the ingested ivermectin was not absorbed and transited directly into faeces, providing a major contribution (82%) to the excretion of parent drug in the dung ($72 = (0.55 / 0.76) \times 100; 82 = (0.55 / 0.67) \times 100$; see Tables 1 and 3).

Individual time profiles of the ingestion rate constant, $Ka$, were generated by modeling and are presented in Fig. 5A. Peak values of $Ka$ were reached between 3 and 16 days after pour-on application. In general, high $Ka$ peak values were associated with a rapid decrease of $Ka$, whereas lower $Ka$ peak values were associated with a slower decrease of $Ka$. Half of the ingestion was achieved between 3 and 7 days post-administration, and 90% of the ingestion was achieved between 9 and 17 days post-administration. The differences observed among cattle are consistent with the large variability expected from the licking behaviour of animals.

**Simulations of ivermectin ingestion profiles with increased rates of percutaneous absorption**

Ingestion profiles were simulated for different magnitudes of percutaneous absorption rates, using the final estimated parameters obtained for each pair of twins. The results of the simulations are presented in Fig 5B for a representative pair of twin cattle. They indicate that the rate constant of percutaneous absorption, $K51$, must be increased by a factor 6 - 10 to obtain less ivermectin ingested by licking than absorbed through the skin, and by at least a factor 50 to reduce the ingestion of ivermectin by one order of magnitude (down to $8 \pm 2.3\%$ of the dose). A 8-fold higher rate constant of percutaneous absorption resulted in a higher bioavailability of topical drug via the skin ($48 \pm 6.4\%$ instead of $10 \pm 2.7\%$), and in lower amounts of ivermectin remaining as a cutaneous depot or degraded ($8 \pm 7.4\%$ of the dose instead of $13 \pm 12.4\%$).

**Simulation of the contamination of non-treated animals with ivermectin by allo-licking**

In the case of multiple oral uptake (modelised by $Ka$), plasma concentrations of 1 ng/mL were achieved following total ingestion of 2 - 3.7% of the pour-on dose in 4 of the 6 pairs of twins, and of 10-11% in the two other pairs. This resulted in detectable (> 0.01 ng/mL) and persistent (> 44 days) concentrations of ivermectin in milk, with maximal values of 0.8 ng/mL. Only 0.02 - 0.11% of the dose had to be ingested in total to recover detectable concentrations of ivermectin in plasma (0.01 ng/mL). In the case of single oral uptake, plasma concentrations of 1 ng/mL
(0.8 ng/mL in milk) were achieved following total ingestion of 0.3 - 2.5% of the pour-on dose in the 6 pairs of twins. Total ingestion of 0.003 - 0.025% of the pour-on dose was sufficient to recover detectable concentrations of ivermectin in plasma.

Figure 5. Ingestion of ivermectin following topical application to cattle along the dorsal midline. A) The rate constant of ingestion, Ka, obtained by modeling is plotted as a function of time, for each of the 6 licking cattle. B) Simulations of the percutaneous absorption (normal-width line) and ingestion (thick line) of topical ivermectin with increased rates of percutaneous absorption in one representative licking cattle. Solid line, control values. Short-dashed line, percutaneous rate multiplied by 8. Medium-dashed line, percutaneous rate multiplied by 50.
Discussion

In contrast to what could be expected for a "percutaneous" formulation, the present study shows that the main route for the systemic absorption of topical ivermectin is not percutaneous but oral, as a result of cattle licking behaviour. This is the first time such an interaction between the individual/social behaviour of animals and the pharmacokinetics of parenterally-administered drugs is reported.

The selected pharmacokinetic model adequately describes the experimental plasma and faecal data observed after i.v. and pour-on administrations of ivermectin in licking and non-licking cattle. The predicted values for the absolute bioavailability of pour-on ivermectin (F_{tot}) and for the drug total clearance (CL_{tot}) are very close to those obtained by non-compartmental analysis. It was assumed that the rate constant of percutaneous absorption, K51, did not differ between lickers and non-lickers. This is supported by a non-significant difference in the estimates of K51_{L} and K51_{nL} when considered as separate parameters in the model, which suggests that the absence of licking for 44 days did not impair the process of ivermectin absorption through the skin.

It is worthwhile to mention that the pour-on data obtained in lickers were totally misfitted by a model containing a single gastrointestinal compartment pooling compartments GIT 1 and GIT 2. Selection of a model with two separate gastrointestinal compartments suggests that the local disposition of ivermectin is not the same at all sites of the gastrointestinal tract in terms of (re-)absorption and elimination. This is in line with the experimental finding of a different ivermectin bioavailability in the rumen and in the abomasum in sheep (Prichard et al., 1985). The low bioavailability of ivermectin after intraruminal administration (75% lower than after intra-abomasal administration) was first attributed to an extensive degradation or metabolisation of drug in the rumen (Prichard et al., 1985). This explanation is however difficult to conciliate with our results, since the unabsorbed fraction of ingested ivermectin was fully recovered in the faeces of lickers. Moreover, several studies argue for the stability of ivermectin in rumen fluids in cattle and in sheep (Bogan and McKellar, 1988; Andrew and Halley, 1996), and show that there is rather an extensive adsorption of ivermectin to the digesta particulates of the rumen (Ali and Hennessy, 1996). Our model stipulates that the fraction of drug which is not absorbed in compartment GIT 1 will not be absorbed in compartment GIT 2. A possible interpretation is that ivermectin remains adsorbed to the particulate phase of the digesta during intestinal transit, and thus is not available anymore for absorption in intestinal fluids. Such a situation has already been described for other compounds such as phenylbutazone (Bogan et al.,
1984; Lees et al., 1988), and is consistent with the high organic-carbon binding constant of ivermectin (K_{oc} = 12600-15700; Halley et al., 1989) and its high hydrophobicity (Pouliot et al., 1997). Ivermectin oral bioavailability was estimated from the model to 28 ± 13.2%, which is in line with the bioavailability found by Chiu et al. (1990) in cattle for an intraruminal bolus of ivermectin relative to the subcutaneous route (26%). However, the contribution of a buccal absorption of drug cannot be excluded.

Pharmacokinetic modeling showed that approximately 76% of the pour-on dose was ingested by licking. As indicated by preliminary deconvolution studies, the rate constant of ingestion, Ka, could be modelised as a biexponential function of time, with an increase up to a maximum followed by a gradual decrease. Ivermectin is a highly lipid soluble drug (Fisher and Mrozik, 1989), which markedly accumulates in the ear wax of pigs (Scott and McKellar, 1992). It is thus possible that ivermectin spreads over the skin of cattle by diffusion within the skin lipid layer, as shown for the antiparasitic agents cypermethrin (Jenkinson et al., 1986), flumethrin (Stendel et al., 1992) and parathion (Brimer et al., 1994) in sheep, cattle or pigs. The first increase of Ka would then be explained by a higher licking efficiency, due to a progressive increase in skin surface exposure. Conversely, the decrease of Ka would correspond to a reduction in licking efficiency, consistent with the decrease of ivermectin concentrations on the skin. This decrease would be more or less rapid depending on individual licking activity. Kinetic modeling shows an ingestion of topical ivermectin up to 19 days post-administration, suggesting that ivermectin was available at the skin surface for a long period of time. This is in agreement with previously published results, indicating that ivermectin was still present on the skin of non-licking cattle 44 days post-administration (Laffont et al., 2001). It is though possible that ivermectin partitions into the various layers of the skin as for other lipid-soluble compounds (chlorpyrifos, Griffin et al., 2000), and is released over a prolonged period at the skin surface. Distribution of the drug in the skin after systemic absorption cannot be excluded, given the high concentrations of ivermectin found in the epidermis and dermis of cattle after subcutaneous administration (Lifschez et al., 2000).

The model indicates that 50-77% of ivermectin absorption in plasma was achieved by the oral route, compared to 23-50% by the percutaneous route. This infers that the systemic exposure of animals most likely depends on their ability to lick themselves (self-licking) or each other (allo-licking). This would explain the erratic bioavailability of pour-on ivermectin observed in the licker group (coefficient of variation of 55% in lickers vs 29% in non-lickers after application of PO2) and in previous studies (Gayrard et al., 1999), as well as the large intra-
individual and within-pair variability of plasma exposure (up to 230% and 70%, respectively) which could not be of genetic origin. More generally, the extent of licking depends on various social, nutritional, physiological, pathological, environmental and managerial factors (Sato et al., 1991, 1993), which makes the delivery of drug even more unpredictable. An increased licking activity has been reported in parasitised cattle (Sato et al., 1991), which could be in favour of a higher plasma exposure of the animals and a better efficacy of the pour-on treatment. On the other hand, potential systemic underexposure could result in subtherapeutic plasma concentrations, which may promote the development of drug resistance (Geerts and Gryseels, 2000). At last, the results question the relevance of performing in vivo bioequivalence assays for evaluation of topical drug formulations in licking animals.

For simplicity reasons, allo-licking was not taken into account in the model. However, although the cattle of the licking group were housed in individual boxes, contacts by allo-licking were observed during the experiments between the immediate neighbours. In one pair of twins, the pour-on dose (500 µg/kg) applied on the back of the licker did not provide a sufficient amount of ivermectin to explain the high faecal data (no ivermectin was left on the skin). This suggests a supplementary source of topical drug consistent with allo-licking.

Under field conditions, allo-licking may result in the contamination of the non-treated cattle in contact with those topically treated. Our simulations (multiple oral uptake) showed a significant (detectable) plasma exposure of non-treated animals which would lick small amounts of ivermectin from the skin of one or several treated cattle (0.02-0.11% of the pour-on dose in total). It is noteworthy that a continuous licking corresponding to 2-11% of the pour-on dose in total would result in detectable concentrations in the milk of non-treated cattle for more than 44 days, with maximal plasma concentrations of 1 ng/mL. These amounts depend obviously on the individual licking activity (traduced by the Ka function), but are all very low compared to the 76% of the dose actually ingested by the treated cattle under the same conditions. Altogether, these results suggest an easy contamination of non-treated cattle by social contact with treated animals and elicit concern over possible insecticide residues in their milk and edible tissues.

The skin provides an excellent barrier against the environment and foreign substances, and many drugs used in human and veterinary medicine exhibit a low rate and extent of dermal absorption (Barry, 2001; Magnusson et al., 2001; Riviere and Papich, 2001). In the present study however, we show that a low rate of absorption through the skin may not ensure a controlled and optimised delivery of drug to the systemic or local circulation. In control cattle, licking reduced by a
factor two the total amount of ivermectin delivered to the plasma via the skin (from 20% of the dose in non-lickers to 10% in lickers) and led to an unpredictable absorption of topical drug by the oral route. As indicated by the simulations, the rate constant of percutaneous absorption $K_{51}$ would need to be increased by a factor of at least 50 to warrant a good percutaneous absorption of drug, independently of animal licking behaviour or other external events (evaporation, accidental removal of the drug …).

In conclusion, we demonstrate that the social/individual licking behaviour of animals can markedly interfere with the disposition of topical drugs and can contribute to a higher excretion of active compound into the environment. We also suggest that allo-licking can result in drug exposure of non-treated animals. This route of contamination has to be considered as a general risk for topically-applied drug formulations in food-producing animals.
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Pharmacokinetic modeling of the disposition of topical ivermectin in cattle


