Feasibility of the Ussing chamber technique for the determination of in vitro jejunal permeability of passively absorbed compounds in different animal species

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The aim of this study was to assess the feasibility of the Ussing chamber technique for the determination of the jejunal permeability of passively absorbed, high permeability model compounds (acetaminophen and ketoprofen) in different animal species. Additionally, electrophysiological measurements and histological examination of pre- and post-incubation tissue specimens were performed. Apparent permeability coefficients of turkey and dog jejunum were low and highly variable due to tissue fragility caused by differences in thickness of the remaining intestinal layers after stripping and resulting in severe damage. Pig and horse jejunum were markedly more suitable for permeability determinations and mild signs of deterioration were noticed after 120 min of incubation. Transepithelial electrical resistance and potential difference did not correlate well with the observed tissue damage. From these data, the Ussing chamber technique appears to allow for permeability measurements within a species, but seems unsuitable for interspecies permeability comparison. However, further validation of the method with low permeability compounds and actively transported compounds is needed.

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INTRODUCTION

A sufficiently high bioavailability is an important prerequisite for most orally administered drugs. Bioavailability is a function of the extent and rate of intestinal absorption (being dependent on the stability of a drug in the gastrointestinal tract, its aqueous solubility and its permeability through the intestinal membrane) on the one hand, and intestinal or hepatic first-pass elimination on the other (Chan & Stewart, 1996). In veterinary product development, researchers are confronted with a wide variety of animal species, differing in anatomy, physiology and biochemistry. The biopharmaceutics classification system (BCS), originally developed as an aid in human drug discovery, defines solubility and intestinal permeability of an orally administered drug as the fundamental properties determining its rate and extent of absorption (Amidon et al., 1995). Similarly, the development of a veterinary BCS, where compounds are categorized under different classes based on in vitro permeability measurements, would allow for predictions of in vivo intestinal permeability. Various biological models are available for the determination of permeability of compounds through intestinal epithelium of animals. The advantage of the Ussing chamber technique using excised intestinal tissue sheets resides in the fact that it is relatively straightforward and species specific. It approaches the in vivo situation more accurately than cell culture–based models, owing to the presence of adequate paracellular permeability, an apical mucus layer, active transport proteins and drug-metabolizing enzymes (Annaert et al., 2000; Castella et al., 2006). Other advantages are the possibility of studying regional differences in drug absorption along the intestine and bidirectional drug transport, the small amount of drug needed (mg quantities) and the analytical cleanliness of the samples (Balimane et al., 2000). Moreover, commercial intestinal epithelial cell lines have not yet been established for all
animal species. In contrast to in situ intestinal perfusion, time-consuming surgery and anaesthesia are avoided. Disadvantages of the technique are the limited viability of the tissue sheets (up to 120 min), the required experience in delicate tissue preparation (Barthe et al., 1998), the lack of blood and nerve supply (Balimane et al., 2000) and the dependence on experimental conditions causing a high interlaboratory variability of results (Nigsch et al., 2007). Thus far, it has been most frequently employed for intestinal transport studies of drugs in mice, rats, rabbits and pigs in an attempt to predict intestinal absorption in humans. One preliminary Ussing chamber study with equine jejunum regarding a BCS for horses yielded promising results (Davis et al., 2006). Permeability (P_{app}) of four drugs with a wide range of physicochemical properties and bioavailabilities in the horse correlated positively with the in vivo bioavailability (R^2 = 0.63) and the log P (R^2 = 0.65) and negatively with the molecular weight (R^2 = 0.85). Permeability was ranked as follows: metronidazole > fluconazole > marbofloxacin > cephalaxin. However, possible tissue toxicity of metronidazole may have falsely increased its permeability. When this molecule was excluded from the regression analysis, both positive correlations became much stronger. The low permeability of cephalaxin may indicate a lack of active transporters that are found in other species. In vitro permeation experiments with excised animal intestinal tissue are part of the recommended methods for permeability determination in the existing BCS Guidance of the U.S. Food and Drug Administration (Center for Drug Evaluation and Research).

In search of frequent and species-specific causes of differences in oral bioavailability and pharmacokinetics, we have previously administered acetaminophen (APAP) and racemic ketoprofen (KTP), a BCS class I (high-solubility, high-permeability) and class II (low-solubility, high-permeability) model compound, respectively, to different monogastric animal species (Neirinckx et al., 2010a,b). Both high-permeability compounds are passively absorbed throughout the small intestine (Rawlins et al., 1977). A trend was observed towards a low bioavailability in chickens (APAP, 42.2%; R(-) KTP, 31.5%; S(+) KTP, 52.6%), turkeys (APAP, 39.0%; R(-) KTP, 42.6%; S(+) KTP, 32.6%) and dogs (APAP, 44.5%; R(-) KTP, 33.6%; S(+) KTP, 89.1%) and a high bioavailability in pigs (APAP, 75.5%; R(-) KTP, 85.9%; S(+) KTP, 83.5%) and horses (APAP, 91.0%). This has been mainly attributed to the higher tendency for first-pass metabolism in poultry and dogs and partially to the fast gastrointestinal transit, reducing time available for absorption, of the former species. Intrinsic permeability of passively absorbed compounds is believed to be equal across species, because the structure of the intestinal epithelium is quite similar (Balimane et al., 2000; Pekkonen et al., 2001). It is our intention to determine the in vitro apparent permeability of APAP and both enantiomers of KTP through jejunal tissue of the previously mentioned animal species. Therefore, the feasibility of the Ussing chamber technique for species permeability determination and comparison will be evaluated by means of electrophysiological measurements and histological examination of pre- and postincubation tissue specimens.

MATERIALS AND METHODS

Animals

Clinically healthy turkeys (BUT Big 6, 6.9 ± 0.7 kg, n = 6), dogs (different breeds, 23.6 ± 8.3 kg, n = 6), pigs ((Landrace × large White × Seghers hybrid synthetic line) × Piétrain, 19.6 ± 1.3 kg, n = 6) and ponies (Shetland, 121.8 ± 2.6 kg, n = 5) of both sexes were included in this study. All animals received a commercial feed, supplemented by hay in case of the ponies. Tap water was provided ad libitum. The animals were fasted overnight before euthanasia.

Drugs and reagents

Crystalline APAP (Bula, Uitgeest, The Netherlands) and racemic KTP (Kela, Hoogstraten, Belgium) were both Ph. Eur. grade. Krebs-Henseleit buffer and fluorescein isothiocyanate-dextran (FITC-dextran) used in the Ussing chamber experiments were purchased from Sigma-Aldrich (Bornem, Belgium). Stock solutions (1000 µg/mL) of APAP and KTP and their respective internal standards ketoprofen (Kela) and fenoprofen (Sigma-Aldrich) used in high-performance liquid chromatography (HPLC) were prepared in HPLC grade methanol (VWR, Leuven, Belgium) and stored at −20°C and renewed monthly to ensure stability. HPLC grade solvents included water, methanol, acetonitrile (VWR) and 2-propanol (Merck, Darmstadt, Germany). Glacial acetic acid (Merck) and triethylamine (Sigma-Aldrich) were of analytical grade.

Ussing chamber experiments

A total of 18 (horses: 15) measurements was taken for each drug (three chambers per animal). Immediately after euthanasia with T61® (Intervet, Mechelen, Belgium), a large segment of midjejenum was collected and immersed into oxygenated (O2/CO2, 95/5) Krebs-Henseleit buffer pH 7.4 (NaCl, 118 mM; CaCl₂, 2.5 mM; KCl, 4.7 mM; KH2PO4, 1.2 mM; MgSO4, 1.2 mM; NaHCO3, 25 mM; and D-glucose, 11.1 mM) at 4°C. Before opening of the intestine, tunicia mucosa and tecta submucosa were stripped of underlying muscle layers (tunicia muscularis) and tunicia serosa by blunt dissection with fine scissors and tweezers. Segments were opened along the mesenteric border and rinsed from intestinal contents with Krebs buffer. Areas containing Peyer’s patches were avoided. Segments of 2.5 cm were cut off, and each was mounted in six modified Ussing chambers (Mueller Scientific Instruments, Aachen, Germany) with an exposed surface area of 1.07 cm². Edge damage was minimized by placing silicon sheets on both sides of the tissue. All tissues were mounted within 20 min following euthanasia. Both mucosal (donor) and serosal (acceptor) compartments were simultaneously filled with 7 mL warm (38°C) Krebs buffer. Solutions were circulated by gas lift (O2/CO2, 95/5) and maintained at 38°C by water-jacketed reservoirs. After an equilibration period of 30 min, 7 mL of a 10.74 mM (1623.4 µg/mL) APAP and a 1.60 mM (405.6 µg/mL) KTP solution in Krebs buffer were
added to the mucosal compartment by replacing the buffer solution. To prevent precipitation, concentrations were lower than the maximal aqueous solubility of both substances at pH 7.4 (Neirinckx et al., 2006). Every 30 min, during 120 min of incubation, 400 μL samples was collected from both compartments. Samples were frozen at −20°C until analysis. During the experiment, transepithelial potential difference (PD, mV) and transepithelial electrical resistance (TEER, Ω·cm²) were monitored as measures of tissue viability and integrity with the tissue unclamped in open circuit mode. Four Ag/AgCl electrodes were connected to each chamber by 3 m KCl-agar bridges. The electrodes were coupled to an external six-channel microcomputer-controlled voltage/current clamp. TEER was determined from voltage deflections in response to bipolar 50- μA current pulses generated for 200 ms and subsequently calculated from Ohm’s law. Data were transferred to a computer with the software package Clamp version 2.14 (Mueller Scientific Instruments). Data were corrected for the offset potential and fluid resistance of the buffer solution which were recorded prior to the experiments. Additionally, a 1 mg/mL FITC-dextran (4 kDa) solution in Krebs buffer was added to the mucosal compartment at the beginning of the experiment as a macromolecular marker of tight junction damage and resulting leakage. Fluorescence was measured at each sampling point by a Fluoroskan Ascent FL microscope (Jeol Europe BV, Zaventem, Belgium).

Permeability calculations

The apparent jejunal permeability coefficient (P_app) was calculated for each drug from the following equation:

\[ P_{app} = \frac{dC}{dt} \times \frac{V}{A \times C_0} \text{ (cm/s)} \]

where \( \frac{dC}{dt} \) is the change in acceptor concentration between 30 and 120 min (μg/mL/s) calculated from the slope of the concentration-time curve, V is the buffer volume in the donor compartment (mL), C_0 is the initial drug concentration in the donor compartment (μg/mL) and A is the exposed tissue surface area (cm²).

Sample analysis

All samples were analysed by HPLC-UV. Equipment and chromatographic conditions were identical to those previously described for the determination of APAP (Neirinckx et al., 2010a) and the enantiomers of KTP (Neirinckx et al., 2010b) in plasma of animals. Samples were ultracentrifuged at 13 000 g during 10 min prior to analysis to achieve precipitation of debris. Next, 100 μL of undiluted samples of the acceptor compartment and 1:1000 (APAP) or 1:20 (KTP) diluted samples in Krebs buffer of the donor compartment were spiked with internal standard, vortexed and injected directly into the chromatographic system. Linear calibration curves (R² > 0.99) for APAP in Krebs buffer were obtained for the acceptor and donor samples in the 0.0125–0.25 μg/mL and 5–20 μg/mL concentration range, respectively. For KTP, linear calibration curves were obtained for the acceptor and donor samples in the 0.025–0.25 μg/mL and 5–20 μg/mL concentration range, respectively. The goodness-of-fit coefficient was <10% for all calibration curves. The accuracy and precision fell within the range of −20 to +10%. The limit of quantification (LOQ) was set at 0.0125 μg/mL (APAP) and 0.025 μg/mL (KTP). All values lower than the LOQ were excluded from the permeability calculations. Concentrations out of the upper limit of the calibration curve were re-analysed after appropriate dilution with Krebs buffer.

Histology

Tissue samples were taken for histological examination before (unstripped and stripped) and after 120 min of incubation in the Ussing chambers. Specimens for light microscopical analysis were placed in phosphate-buffered 3.5% formaldehyde for 24 h and embedded in paraffin by an automated system (Microm STP 4200 Tissue Processor, Prosan, Merelbeke, Belgium). Sections of 8 μm were cut (Microm HM360), deparaffinized in xylene, rehydrated in descending grades of alcohol and stained with haematoxylin-eosin (H.E.). After dehydration in ascending grades of alcohol and xylene, sections were mounted with DPX (DPX Laboratory Supplies, Poole, UK). Sections were analysed using a BX61 motorized research microscope (Olympus Belgium NV, Aartselaar, Belgium). Additionally, comparative measurements of the villus length and thickness of lamina propria, lamina muscularis mucosae, tela submucosa and tunica muscularis were taken using the Cell-F software (Olympus Belgium NV). Values are expressed as mean of eight randomly chosen areas.

For scanning electron microscopy, samples were prefixed in a HEPES-buffered 2% paraformaldehyde-2.5% glutaraldehyde solution for 24 h. Postfixation in an un-buffered 1% osmium tetroxide solution was followed by dehydration in ascending grades of acetone. After critical point drying with CO₂ (CDP 030; Balzers, Sercobal, Merksem, Belgium), specimens were mounted, platinum coated (JFC-1300 Autofine Coater, Jeol, Tokyo, Japan), and examined using a Jeol JSM 5600 LV scanning electron microscope (Jeol Europe BV, Zaventem, Belgium).

Statistical analysis

Data were statistically analysed by means of single-factor analysis of variance (ANOVA), using SPSS 15.0 software for Windows. For those parameters that were not normally distributed, the Kruskal–Wallis ANOVA on ranks was used. Multiple comparisons of mean were performed using the Schefé test. A value of P < 0.05 was considered significant.

RESULTS

Jejunal permeability

For each compound, the concentration in the donor compartment remained stable over time, indicating the absence of
Striking differences in $P_{app}$ values were found between species. Overall, pig jejunal preparations displayed the highest permeability for all compounds, followed by horse tissue preparations. Permeability of turkey and dog intestinal sheets was low and highly variable. Within each species, no permeability differences could be demonstrated between both enantiomers of KTP. In horses, however, APAP appeared significantly more permeable than R(-) KTP and S(+) KTP. For all three molecules, no differences in $P_{app}$ could be shown between turkey and dog jejunum. Permeability of pig jejunum was significantly higher in all cases. Although not always significant because of the high variability of turkey and dog permeability, $P_{app}$ values of horse tissue were generally higher than those of the former two species and lower than those of pigs.

A time-dependent increase in the measured optical density values (data not shown) of FITC-labelled dextran at the different time points caused by normal tight junction deterioration was noticed. Chambers displaying values higher than 1.5 (arbitrary units of optical density) because of leakage were excluded from permeability calculations.

**Electrophysiological measurements**

The course of the electrical parameters TEER and PD is shown in Tables 2 and 3, respectively. Dog intestinal preparations showed the highest TEER with a mean value of about 260 W·cm², followed by horse (170 W·cm²), turkey (80–150 W·cm²) and pig (70 W·cm²) jejunum. Only in turkey jejunum, a modest decline in TEER over experimental time could be noticed. Mean TEER values of the other species remained constant.

Mean PD of pig and dog jejunal preparations varied between 1 and 3 mV and declined with time, while in turkey jejunum values were as high as 5–6 mV, exhibiting a less pronounced decline. Mean PD of horse jejunum increased from 6 mV to nearly 13 mV over the experimental time.

**Histology**

Morphological integrity of the jejunal tissue of the different species was interpreted by means of the light microscopic appearance of the H.E-stained epithelium at different time points (Fig. 1).

The first column of Fig. 1 shows unstripped tissue, taken immediately after euthanasia of the animal. Although the general structure is similar across species, certain specificities in the thickness of different layers are visible. In the second column, the appearance of the stripped intestinal preparations just before mounting in the Ussing chambers is displayed. Striking differences are obvious between pig and horse preparations where a substantial part of the tela submucosa is left in place and dog and turkey preparations where only the thin lamina propria mucosae and lamina muscularis mucosae as a part of the tunica mucosa are left at the serosal side. After 120 min of incubation in the Ussing chambers (third and fourth column), villi became damaged to various degrees depending on the species, while crypts remained intact. Cell debris and mucus accumulated on the luminal surface in all specimens. Oedema in pig and to a lesser extent in horse jejunum is characterized by a dilatation of the central lacteal in the villi, an increased distance between glands and cells in the lamina propria mucosae and a separation of submucosal collagen bundles. A moderate degree of epithelial cell release from the lamina basalis at the villus tips is visible. A total disorganization of the normal structure occurred in turkey and dog preparations. The epithelium and tight junctions were largely disrupted, especially at the villus tips of dog jejunum and the normal parallel orientation of the villi of turkey jejunum has become unrecognizable.

### Table 2. Mean (±SD) changes in TEER over turkey, dog, pig and horse jejunum during 120-min experimental time determined by the Ussing chamber technique

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Turkey</th>
<th>Dog</th>
<th>Pig</th>
<th>Horse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>148.3 ± 43.8</td>
<td>252.4 ± 83.8</td>
<td>66.7 ± 23.2</td>
<td>175.3 ± 146.1</td>
</tr>
<tr>
<td>30</td>
<td>113.0 ± 10.6</td>
<td>261.4 ± 90.0</td>
<td>70.3 ± 29.2</td>
<td>157.7 ± 120.8</td>
</tr>
<tr>
<td>60</td>
<td>103.0 ± 21.0</td>
<td>250.9 ± 109.2</td>
<td>68.7 ± 32.5</td>
<td>157.3 ± 87.6</td>
</tr>
<tr>
<td>90</td>
<td>78.5 ± 45.5</td>
<td>263.3 ± 113.4</td>
<td>68.3 ± 32.7</td>
<td>169.5 ± 82.1</td>
</tr>
<tr>
<td>120</td>
<td>90.0 ± 50.0</td>
<td>257.9 ± 102.8</td>
<td>68.7 ± 33.7</td>
<td>180.0 ± 81.9</td>
</tr>
</tbody>
</table>

TEER, transepithelial electrical resistance.

### Table 3. Mean (±SD) changes in PD over turkey, dog, pig and horse jejunum during 120-min experimental time determined by the Ussing chamber technique

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Turkey</th>
<th>Dog</th>
<th>Pig</th>
<th>Horse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.2 ± 3.4</td>
<td>2.9 ± 2.0</td>
<td>2.6 ± 1.2</td>
<td>5.8 ± 3.2</td>
</tr>
<tr>
<td>30</td>
<td>5.3 ± 2.1</td>
<td>1.7 ± 1.2</td>
<td>2.5 ± 1.2</td>
<td>11.0 ± 4.7</td>
</tr>
<tr>
<td>60</td>
<td>5.2 ± 2.5</td>
<td>1.5 ± 0.9</td>
<td>2.0 ± 1.0</td>
<td>12.5 ± 4.5</td>
</tr>
<tr>
<td>90</td>
<td>5.0 ± 2.7</td>
<td>1.1 ± 1.3</td>
<td>1.5 ± 0.9</td>
<td>10.6 ± 3.3</td>
</tr>
<tr>
<td>120</td>
<td>4.9 ± 2.8</td>
<td>1.3 ± 0.9</td>
<td>1.1 ± 0.9</td>
<td>12.9 ± 3.7</td>
</tr>
</tbody>
</table>

PD, potential difference.
Scanning electron microscopy of the epithelial surface confirmed these findings (Fig. 2). Despite the presence of debris and mucus between the villi after 120 min, villi retained a normal appearance in sections of pig and horse jejunum, in strong contrast to the disrupted aspect of turkey and dog villi.

In Table 4, the measured villus length and the thickness of different layers of the intestinal wall are summarized. A strikingly higher villus length was measured in turkey specimens. Thickness of the lamina propria mucosae was different in all species, with dog jejunum having the highest and turkey
jejunum the lowest value. The lamina muscularis mucosae were again the thickest in dogs, while the tela submucosa and tunica muscularis were manifestly thicker in horse jejunum. The tunica muscularis of dog jejunum was twice as thick as that of turkeys and pigs, but thinner than that of horses.

**DISCUSSION**

**Jejunal permeability**

We assume that the severe epithelial damage and the accumulation of cellular debris and mucus on the luminal side are responsible for the low and highly variable permeability of all three molecules through turkey and dog jejunum. A previous study similarly describes a significantly lower permeability of compounds through stripped dog small intestinal segments compared to rabbit and monkey tissues (Jezek et al., 1992). Unfortunately, no histological examination of the segments was performed in that study. Despite the faster intestinal transit of dogs (Dressman, 1986), a better in vivo absorption of many drugs compared to humans is observed (Chiou et al., 2000). This is attributed to the longer villi, the presence of more and wider tight junctions facilitating paracellular transport and the higher bile salt secretion rate and concentration (Karari, 1995; He et al., 1998) in dogs. The obtained turkey and dog apparent permeability coefficients in this study are difficult to interpret, in contrast to pig and horse data. KTP enantiomers displayed equal permeability coefficients, which is not unexpected considering their passive absorption and similar physicochemical properties (Caldwell et al., 1988). The aforementioned high and equal bioavailability of both enantiomers after administration of a suspension to pigs confirms this observation. Landoni and Lees (1995) found an extremely low bioavailability after administration of micronized powder in an oil-based paste to horses with restricted access to food (2.67% and 5.75% for R(-) and S(+) KTP, respectively), while bioavailability increased markedly when the same powder was administered in hard gelatin capsules (50.55% and 54.17% for R(-) and S(+) KTP, respectively). A lower degree of dissolution of the oil-based paste in the gastrointestinal tract, illustrating the formulation influence on bioavailability, was held responsible for this phenomenon. Permeability of APAP appeared higher than KTP permeability through pig and horse small intestine, although statistical significance could only be shown for the latter. The weakly acidic pK<sub>a</sub> (4.76) of KTP causing the molecules to be 100% ionized in pH 7.4 possibly impedes passive diffusion through the epithelial cell membranes and connective tissue towards the donor compartment. APAP, having a weakly acidic pK<sub>a</sub> of 9.5, remains unionized, allowing a rapid diffusion. This effect is probably enhanced in vitro because of the absence of absorption promoting factors such as blood supply, peristalsis, bile salts and surfactants. Absorption of high-permeability compounds such as APAP and KTP occurs predominantly at the villus tips (Polentarutti et al., 1999). The excessive villus and villus tip destruction in turkey and dog jejunal preparations might explain the strikingly lower permeability coefficients. The lower P<sub>app</sub> values of the three molecules through horse tissue compared to pig tissue probably arise from the thicker submucosal remainder in horse preparations. The apparent permeability of APAP through pig jejenum corresponds to the previously described value of 8.67 x 10<sup>-6</sup> cm/s through rat jejunum (Watanabe et al., 2004). Based on permeability measurements of different drugs in rat jejunum, ileum and colon, passively absorbed compounds were divided into low- and high-permeability drugs with P<sub>app</sub> values ranging from 0.9–8.3 x 10<sup>-6</sup> cm/s to 11.4–100.3 x 10<sup>-6</sup> cm/s, respectively (Ungell et al., 1998).

Although only three compounds were studied, an attempt was made to correlate P<sub>app</sub> values and in vivo absolute bioavailability for pigs and horses, R<sup>2</sup> values of 0.90 and 1.00 were found between P<sub>app</sub> and bioavailability in horses, using our value of 91% for APAP (Neirinckx et al., 2010a) and the bioavailability values reported by Landoni and Lees (1995) for R(-) and S(+) KTP. Unfortunately, a possible lower intestinal permeability of the enantiomers of KTP in horses could not be distinguished from other factors, such as first-pass metabolism and the interference of efflux transporters, known to inhibit the absorption of xenobiotics in horses.

It is also worth mentioning that some compounds may interact with the barrier function of the gastrointestinal epithelium, as has been previously observed with acetylsalicylic acid (Oshima et al., 2008) and mycotoxins (Pinton et al., 2009). In both cases, a decrease in TEER and an increase in dextran permeability were observed in human and porcine gastric and intestinal epithelial cell lines, respectively. The Ussing chambers

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**Table 4.** Villus length and thickness (μm) of the intestinal layers of different animal species (mean ± SD). From the luminal to the serosal side, intestinal layers comprised the lamina propria mucosae (L. propria muc.), lamina muscularis mucosae (L. musc. muc.), tela submucosa (T. submucosa) and tunica muscularis (T. muscularis)

<table>
<thead>
<tr>
<th>Species</th>
<th>Villus</th>
<th>L. propria muc.</th>
<th>L. musc. muc.</th>
<th>T. submucosa</th>
<th>T. muscular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>1668 ± 467.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>122 ± 25.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65 ± 5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>711 ± 22.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dog</td>
<td>668 ± 104.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>616 ± 159.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>126 ± 17.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>225 ± 16.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1429 ± 237.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pig</td>
<td>585 ± 108.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>479 ± 70.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28 ± 6.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>241 ± 26.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>648 ± 45.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Horse</td>
<td>390 ± 104.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>266 ± 39.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>102 ± 15.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>293 ± 136.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2090 ± 287.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Within a column, values having different superscript letters are significantly different (P < 0.05). n.d.: not detected.
Electrophysiological measurements

The stable or slightly decreasing TEER values did not seem to correspond with the observed severe morphological damage in dog and turkey intestinal sheets and showed a poor correlation with the thickness of the mounted segments. Polentarutti et al. (1999) claim that TEER does not reflect tissue integrity alone and different, conflicting effects add up to lead to a constant TEER value. Analogous findings of Soderholm et al. (1998) were assigned to an increase in TEER owing to submucosal oedema, annihilating the decrease owing to damaged tight junctions. The markedly higher TEER of dog jejunal preparations is in accordance with the findings of Takeyoshi et al. (2001) and could be caused by the assumed higher frequency of tight junctions in dogs (He et al., 1998; Chiou et al., 2000). TEER values of pig jejunal tissue fell within the generally observed range (Moeser et al., 2007). Compared to pig jejunum, TEER of horse jejunum proved to be twice as high, which could be attributable to the significantly thicker submucosal remainder.

As expected, PD values of turkey, dog and pig jejunum decreased with experimental time because of a decreasing viability and ion pump activity of the intestinal preparations. PD of pig jejunum evolved in the same range as described by Nejdors et al. (2000). However, PD values were not well correlated with the morphological appearance of the tissue of the different species. In the study of Soderholm et al. (1998), the majority of specimens with advanced histological changes also did not show clear signs of poor viability. Bajka et al. (2003) demonstrated through the capacity of rat ileal tissue to maintain a high and stable PD following scraping of the mucosa that the seromuscular layer alone was able to maintain a stable PD. Reasons for the increase in PD of horse intestinal sheets are unclear. A similar fluctuating pattern was observed by Polentarutti et al. (1999) with rat colon and was assumed to be caused by a much later equilibrium of the tissue segment or a secretion of chloride into the lumen as a response to stressful stimuli.

Histology

Although the wall of the intestine of galliforms (chickens and turkeys) is similar to that of mammals, the extremely thin tela submucosa is a notable difference (Bacha & Bacha, 2000). Consequently, after removal of the seromuscular layers of turkey jejunum, a fragile mucosal preparation was left. Moreover, attempts to strip jejunal tissue of 7-week-old broiler chickens failed because of the inevitable occurrence of ruptures in the mucosal remainder. Leaving the muscular layers in place was not an option, because permeability was extremely low and irreproducible. For reasons of uniformity and comparability of $P_{app}$ values between species, stripped preparations of all species were preferred. Therefore, turkey jejunum was used instead of chicken jejunum. In contrast to pig and horse jejunum, dog jejunal intestinal layers were tightly attached, rendering it difficult to dissect the seromuscular layers without damaging the tissue. Removal of the tela submucosa together with the seromuscular layers proved to be the fastest and least complicated method.

The mild oedema and the lifting of the epithelium from the lamina basalis in pig and horse specimens can be considered as a part of the normal degradation process of the tissue during a 2-h incubation period. The severe morphological deterioration of the villi of turkey and dog specimens was probably caused by the fragility of the thin tissue preparations, in combination with the long and leaf shaped aspect of turkey villi. Analogous to the observations of Inagaki-Tachibana et al. (2008) in mice, villi were almost completely destroyed after 120 min, while crypts remained intact.

In conclusion, the feasibility of the Ussing chamber technique for the in vitro permeability measurement of passively absorbed compounds through pig and horse jejunum was confirmed. Final validation of the method with model compounds covering the full range of low, moderate and high permeability, allowing for cut-off $P_{app}$ values to be established, offers possibilities for the development of a veterinary BCS for each species separately. Considering species differences in the thickness of the remaining layers of the intestinal wall after stripping, we propose that the technique should not be applied for interspecies permeability comparison. Accordingly, we recommend the Ussing chamber technique for a BCS permeability classification of compounds within a species. We were unable to develop a reproducible method for permeability determinations through turkey and dog jejunum owing to tissue fragility and severe damage, which illustrates the importance of a supporting submucosal layer. The obtained low apparent permeability coefficients in those species were not consistent with the known high permeability of APAP and KTP. Further optimization of the stripping method in dogs and poultry is needed. Histological examination of tissue specimens is particularly useful because the electrophysiological parameters PD and TEER do not change proportionally to tissue alterations.

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