Changes in Antibiotic Distribution in Pancreatitis

Kent J Fanning\textsuperscript{1,2}, Thomas A Robertson\textsuperscript{1,3}, Johannes B Prins\textsuperscript{1,4} and Michael S Roberts\textsuperscript{1,3*}

\textsuperscript{1} Therapeutics Research Centre, School of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland, 4102, Australia.
\textsuperscript{3} School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia 5001, Australia.

Present addresses:
\textsuperscript{2} Agri-Science Queensland, Department of Employment, Economic Development and Innovation, Health and Food Sciences, PO Box 156, Archerfield BC Qld 4108 Australia
\textsuperscript{4} Mater Medical Research Institute, Level 3, Aubigny Place, South Brisbane 4101 Australia

*Correspondence: P.O. Box 6067, Buranda, Qld 4102, Australia
Tel +61-411264506; Fax +61-7-32405806 Email: m.roberts@uq.edu.au
Abstract

This work sought to define how pancreatitis affected antibiotic distribution in a perfused rat pancreas model. The distribution kinetics of four antibiotics were examined in control and diseased animals. Meropenem and piperacillin distributed into the extracellular space and their distribution kinetics were unaffected by pancreatitis. In contrast, ciprofloxacin showed a reduced uptake into pancreatitis cells and clindamycin showed a reduced distribution in pancreatitis cells.
Key Words

Pancreatitis; antibiotics; pharmacokinetics; fibrosis; distribution
Although the use of antibiotics in pancreatitis still remains controversial (3, 4, 15), death from bacterial infection is the leading cause of morbidity and mortality in severe acute pancreatitis (SAP) (14). Studies have investigated penetration into diseased human tissue (1, 2, 5, 13), as well as a variety of animal models (6, 9, 10, 16).

However there appear to be few studies examining the rate of antibiotic uptake into pancreatic tissue. To our knowledge, this is one of the few studies investigating the rate of uptake of antibiotics in normal and diseased pancreas and most likely the first in the isolated perfused pancreas. Such a model enables the precise rate of uptake and efflux for antibiotics in the pancreas to be defined, as well as the nature of their distribution in the normal and diseased pancreas.

All procedures involving the animals were carried out with adherence to the University of Queensland Animal Care Committee guidelines (AEC#: PAH/588/06/NHMRC). Pancreatitis was induced by giving male Wistar rats a single injection of 6 mg/kg dibutyltin dichloride (DBTC), via the tail vein, according to a previously described method (16). Rats were fasted from 104 h post injection and then, following the final blood sample at 120 h post injection, anaesthetized as previously described (8). The pancreas perfusions were carried out similarly to the method described previously (8) with a series of 5 individual 20 µl bolus injections (7): piperacillin (2 mg/ml), meropenem (1 mg/ml), ciprofloxacin (200 µg/ml), clindamycin (1 mg/ml) and [14C]-sucrose with [3H]-water (each drug bolus contained [125I]-albumin). The perfusate and outflow profiles were analysed as described previously to obtain the fraction unbound in perfusate \( f_u \), the albumin space \( V_{alb} \).
sucrose space $V_{\text{suc}}$, water space $V_{\text{water}}$, drug distribution volume $V_d$, fraction unbound in tissues $f_{\text{ut}}$, permeation rate constant $k_{\text{in}}$, efflux rate constant $k_{\text{out}}$, and permeability surface area product $(PS)$ (7, 8). Fibrosis was quantified using a method that has been previously utilized for the liver (11).

Histological examination of the diseased pancreas showed pathologies including the formation of ductal complexes with apoptotic and necrotic cells, massive inflammatory cell infiltration, fat necrosis and leukocyte infiltration. The DBTC-treated animals had significantly increased fibrosis index (control 17.6 ± 1.7 %, pancreatitis 0.5 ± 0.2 %, p<0.002), characterized by extensive collagen deposition. The dry/wet ratio of the pancreas preparation was unchanged in the diseased animals.

Similar albumin and sucrose spaces were seen in the control and pancreatitis models but there was a significantly lower water space in pancreatitis (p<0.02) (Table 1). Thus there was no difference in interstitial volume (control 0.15 ± 0.02 ml·g$^{-1}$, pancreatitis 0.16 ± 0.03 ml·g$^{-1}$) but there was a significantly lower cellular volume in the diseased pancreas (control 0.42 ± 0.02 ml·g$^{-1}$, pancreatitis 0.29 ± 0.03 ml·g$^{-1}$, p<0.02).

Figure 1 shows the outflow concentration-time profiles for the antibiotics in the perfused rat pancreas. Also included in Figure 1 are the non-linear regression fits of the physiologically based pharmacokinetic model used. The kinetic parameter estimates derived from these profiles are shown in Table 2. It is evident that piperacillin and meropenem are mainly distributed into the sucrose space (i.e. perfusate + interstitial). In addition, their disposition kinetics appear to be unaffected by the presence of pancreatitis. It is evident that the uptake of ciprofloxacin (as
defined by $PS, k_{in}$ and $k_{in}/k_{out}$) into the pancreas is inhibited by the pancreatitis but that its efflux is relatively unaffected. In contrast, the influx and efflux constants for clindamycin are unaffected by pancreatitis. In addition, the binding of clindamycin in pancreas tissue ($fu_T$) was unchanged (control 0.19 ± 0.03, pancreatitis 0.26 ± 0.04). Hence, as the extracellular (sucrose) space is unchanged, the decrease in $V_d$ for clindamycin arises mainly from a decrease in the cellular space it distributes into in the pancreatitis ($V_d = V_p + fu.V_{water/fu_T}$) (8). This finding is consistent with the change in cellular volume based on the difference in the water and sucrose spaces, as described earlier.

This work suggests that antibiotics with a distribution limited to the pancreas interstitial space are not significantly affected by pancreatitis, consistent with the similar extracellular spaces estimated using sucrose (Table 1). The result for piperacillin agrees with the finding of no change in penetration into diseased tissue as reported previously (19). However the current result for meropenem is in conflict with the work of Saglamkaya et al (17). They investigated animals both 6 h (disease characterized by edema) and 48 h (disease more necrotic) following treatment with combined glycodexoxycholic acid infusion / intravenous cereulein, to induce SAP. Meropenem’s penetration was highest at 6 h ($C_{pancreas} / C_{serum} = 0.93$) and fell slightly, yet was still significantly higher than the control, at 48 h ($C_{pancreas} / C_{serum} = 0.81$ vs control $C_{pancreas} / C_{serum} = 0.33$). Perhaps this penetration would continue to decrease with time as the disease becomes less edematous. Several studies have shown that penetration differences (both increased and decreased) into diseased versus control tissue are more marked in the earlier and/or more edematous forms of acute pancreatitis (9, 10, 17), with evidence suggesting that this is related to capillary
pancreatic blood flow (9). The presence of more severe necrosis in Saglamkaya’s work than in the present model, may also have contributed to the higher penetration ratio at the 48 h time point.

The significantly decreased uptake for ciprofloxacin in pancreatitis may reflect a potential active uptake of this compound into the pancreas and an inhibition of the uptake in pancreatitis due to fibrosis. There are a number of studies demonstrating active uptake of ciprofloxacin across epithelial cells (see for example (21)) however there do not appear to be any studies previously suggesting active uptake for ciprofloxacin in the pancreas. The almost 10 fold higher \( k_{in}/k_{out} \) for this drug that has low albumin binding (\( fu \sim 0.9 \)) but relatively low \( V_d \) suggests that there is an active uptake process. An inverse relationship between \( \log PS \) and fibrosis index for ciprofloxacin (\( r^2=0.68 \)) was seen in the present study, which further suggests active transport as we have shown that fibrosis can reduce \( PS \) for cationic drugs and taurocholate extraction in liver diseases (11, 12). The reduced \( V_d \) for clindamycin attributed to a reduced cellular volume is consistent with chronic pancreatitis being associated with a reduced pancreatic volume (18). A previous study in dogs also observed no significant change in the pancreas/plasma concentrations of clindamycin in SAP (20).

In conclusion, the tissue kinetics of meropenem, piperacillin, ciprofloxacin and clindamycin have been described in the perfused rat pancreas of both control and pancreatitic animals. The disposition of meropenem and piperacillin appear relatively unaffected by the pancreatitis, whereas the uptake of ciprofloxacin is inhibited and there is a reduced distribution of clindamycin into pancreatitic cells, consistent with an observed reduction in cellular volume in pancreatitis.
Acknowledgements

This work was financially supported by a grant from the National Health and Medical Research Council of Australia and a University of Queensland Development grant.

Thanks goes to the following people for their valuable assistance: Megan Bathurst and Tracy Millard (animal handling), Alexander Klentzos and Yuhong Zou (perfusions), Ben Winkle (HPLC), and Clay Winterford, Glenda Gobe and Leila Cuttle (histology).
Abbreviations

1. albumin space - $V_{alb}$
2. apparent volume of distribution - $V_d$
3. concentration of drug in pancreas – $C_{pancreas}$
4. concentration of drug in serum – $C_{serum}$
5. dibutyltin dichloride – DBTC
6. molecular weight – $MW$
7. octanol water partition coefficient at pH 7.4 – $\log P_{7.4}$
8. permeability rate constant from the extravascular to the vascular space - $k_{out}$
9. permeability rate constant from the vascular space to the extravascular space - $k_{in}$
10. permeability surface area product - $PS$
11. severe acute pancreatitis – SAP
12. sucrose space - $V_{suc}$
13. unbound fraction in perfusate – $fu$
14. unbound fraction in tissue - $fu_T$
15. water space - $V_{water}$
References


Table

Table I. Distribution Volumes of Albumin, Sucrose and Water in the Perfused Rat Pancreas (Mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Control animals (n=5)</th>
<th>Diseased animals (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{alb}}$ (ml·g$^{-1}$)</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>$V_{\text{suc}}$ (ml·g$^{-1}$)</td>
<td>0.30 ± 0.03</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>$V_{\text{water}}$ (ml·g$^{-1}$)</td>
<td>0.72 ± 0.03</td>
<td>0.60 ± 0.01* (p&lt;0.02)</td>
</tr>
</tbody>
</table>

$V_{\text{alb}}$ = albumin space, $V_{\text{suc}}$ = sucrose space, $V_{\text{water}}$ = water space
### Table II. Model Derived Kinetic Parameters and Protein Binding of Various Antibiotics in the Perfused Rat Pancreas (Mean ± SEM)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control Animals (n=5)</th>
<th>Diseased Animals (n=5)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MW</td>
<td>Log P&lt;sub&gt;7.4&lt;/sub&gt;</td>
<td>fu</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td>-------------------</td>
<td>----</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>518</td>
<td>-1.84</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>Meropenem</td>
<td>383</td>
<td>-5.7</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>331</td>
<td>-0.90</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>425</td>
<td>0.40</td>
<td>0.91 ± 0.01</td>
</tr>
</tbody>
</table>

*MW* – molecular weight, *Log P*<sub>7.4</sub> = logarithm of octanol-water partition coefficient at pH 7.4, *fu* = fraction unbound in perfusate, *V*<sub>d</sub> = distribution volume, *k*<sub>i</sub> = permeation rate constant, *k*<sub>o</sub> = efflux rate constant, *PS* = permeability surface area product.
Figure Legend

Figure 1 Typical fit of outflow profile data of antibiotics (A – piperacillin, B-meropenem, C-ciprofloxacin, D - clindamycin) in control (●) and diseased (○) animals. The lines indicate the fitted curves applying a physiologically based pharmacokinetic model.