FATE OF SELECTED CEPHALOSPORINS IN THE ALIMENTARY TRACT OF RATS

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After the establishment of broad-spectrum injectable cephalosporin antibiotics such as cephaloridine and cephalothin in clinical use, analogues effective when given orally were sought. New compounds were screened for potential absorption from the gut by determining the percentage of unchanged substance recovered in the urine after one oral dose of 5 mg had been given to rats, but this gave no information about what happens in the gastro-intestinal tract to a compound that does not appear in any quantity in the urine.

The oral administration of labelled cephalosporins to rats may lead to the appearance of radioactive degradation products in the urine. Numerous cephalosporins were tested in this way (Culp, Marshall and McMahon, 1963; Sullivan and McMahon, 1967; Sullivan, Billings and McMahon, 1969a and b), and all except cephaloxin were excreted in the urine as a mixture of metabolites. The conclusion was drawn that most of these degradation products had been formed in the alimentary tract, but no specific site of degradation was implicated. It was also uncertain whether a poorly absorbed cephalosporin was one that was degraded so quickly that absorption could not occur, or whether slow absorption resulted in degradation by prolonging the stay of the antibiotic in the gut. In the present study we attempted to resolve this question.

Materials and Methods

Cephalosporin analogues

Three analogues were chosen to span the possible range of absorption after oral administration (see Ross et al., 1970). These were cephalaxin (CEX) which is very well absorbed, cephaloridine (CER) which is poorly absorbed, and 7-(thiethyl-2'-acetamido)-3-methylceph-3-em-4-carboxylic acid or TMC, which is absorbed to an intermediate extent.

Microbiological assay

The three cephalosporins were assayed by an agar diffusion method as described by Ross et al.

Animals

Female 100-g Albino Wistar rats were used. They were anaesthetised with veterinary Nembutal (Abbott Laboratories) given intraperitoneally in a dose of 50 mg per kg. To assist recovery from this deep anaesthesia, the rats were given bemegride (Megimide, Nicholas Laboratories) subcutaneously (25 mg per kg) after operation.

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Experimental procedures

Experiments in animals with ligated intestines. The rat intestine was divided into four portions for the purpose of the experiments: (1) the stomach; (2) the first 6 in. (15 cm) of the duodenum; (3) the remaining duodenum, jejunum and ileum; and finally (4) the caecum and rectum.

These regions were separated by ligation and 5 mg of cephalosporin in 0.2 ml of 0.05M phosphate buffer—at pH 7 for CER and TMC and at pH 6 for CEX—was given by injection into one of the four regions.

Sixteen rats were used for the study of each ligated region, in two separate groups of eight. After 1 hr the rats were killed, and the ligated gut portions were removed, homogenised in phosphate buffer and assayed microbiologically for residual, i.e., unabsorbed, undegraded cephalosporin. Serum samples were assayed for absorbed cephalosporin. Anaesthesia was maintained during the whole of the experiment.

Experiments in animals with unligated intestines. Anaesthetised and laparotomised rats were given the selected cephalosporin by intraduodenal injection as a 5-mg dose in 0.2 ml phosphate buffer; during injection, the duodenum was temporarily clamped in an attempt to reduce the reflux of cephalosporin into the stomach. The intraduodenal route of administration was chosen to avoid excessive dilution by stomach contents and thus to provide conditions comparable with those in ligated intestine experiments.

Sixteen rats (in two groups of eight) were killed at each time of 0, 1, 2, 4 and 6 hr after injection. Blood samples were taken, and the gastro-intestinal tract together with its contents was removed and separated into the regions described above. Urine samples were collected every 2 hr from rats held in metabolism cages. Urine, serum and gut homogenates were all assayed microbiologically for undegraded cephalosporin.

The anaesthetic and laparotomy may have had some effect on the absorption and excretion of cephalosporins by the rats. The extent of this effect was determined by comparing the rates of recovery of CEX in normal and manipulated animals, after both oral and subcutaneous administration of antibiotic.

RESULTS

Serum half-life of the cephalosporins in the rat

Serum concentrations of CER, TMC and CEX were measured after intraperitoneal administration to rats. The serum half-life of each compound was calculated from the serum level curve over the first 60 min. after injection. The values obtained were 14.6 min., 12.0 min. and 14.0 min. respectively for CER, TMC and CEX.

Recovery of cephalosporins after injection into ligated portions of the alimentary tract of rats

The results of the experiments in which 5 mg of cephalosporin was injected into ligated parts of the gastro-intestinal tract are given in table I. In general, reproducible and consistent serum levels were found only when the antibiotics were injected into the two regions of the small intestine. The majority of rats showed no detectable antibiotic in the serum after injection into the stomach and caecum. The smallest amount of antibiotic detectable in serum by the assay method used was 0.16 µg per ml for CER, 2.0 µg per ml for TMC and 2.5 µg per ml for CEX. After injection into the small intestine, the resulting serum levels for CEX were found to be three to six times higher than those recorded for CER and two to three times higher than those for TMC.
Table I also shows the percentage of antibiotic remaining after 1 hr in the segments of gut into which the injections had been made. This percentage decreased progressively from stomach to caecum and rectum, and the fall was much more pronounced with CER and TMC than with CEX. The greatest loss of CER and TMC occurred in the caecum and rectum; on average only 22 per cent. and 16 per cent. respectively of the initial dose was recovered after 1 hr, but as much as 53 per cent. of the CEX was recovered under the same conditions.

**Table I**

Percentage recovery of antibiotic from the ligated portion of the alimentary tract into which it had been injected* 1 hr before, and serum level of antibiotic after the same time-interval

<table>
<thead>
<tr>
<th>Site of injection and sampling</th>
<th>Cephaloridine</th>
<th>TMC†</th>
<th>Cephalixin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean percentage recovered from portion</td>
<td>Mean (and range) of serum levels (μg per ml)</td>
<td>Mean percentage recovered from portion</td>
</tr>
<tr>
<td>Stomach</td>
<td>80</td>
<td>1·2 (&lt;0·16-3·9)</td>
<td>63</td>
</tr>
<tr>
<td>1st 6 in. (15 cm) of duodenum</td>
<td>72</td>
<td>1·6 (0·72-3·9)</td>
<td>60</td>
</tr>
<tr>
<td>Rest of small intestine</td>
<td>55</td>
<td>3·3 (0·2-10·5)</td>
<td>42</td>
</tr>
<tr>
<td>Caecum and rectum</td>
<td>22</td>
<td>1·6 (&lt;0·16-3·4)</td>
<td>16</td>
</tr>
</tbody>
</table>

* 5-mg dose in anaesthetised rats; 16 rats for each antibiotic and each site of injection, unless stated otherwise.
† TMC = 7-(thienyl-2'-acetamido)-3-methylceph-3-em-4-carboxylic acid.
‡ Average of results for eight rats.

**Recovery of cephalosporins after intraduodenal injection into unligated rat intestine**

Ligation of the rat intestine for 1 hr caused some swelling and discoloration, especially of the duodenum. Since this may have affected the rate of absorption of antibiotics, further tests were done in which 5 mg of cephalosporin was injected into the duodenum without ligation. Table II shows the percentage of this dose that was present in the whole gut and the blood at various times after injection, and the cumulative percentage that had been excreted in the urine up to these times.

The amount of CER and TMC present in the blood was minimal at all times; the amount of CEX was 10–20 times higher, and reached 2·6 per cent. of the dose 2 hr after injection. Recoveries from the urine up to 6 hr after
injection were 2 per cent. for CER, 7 per cent. for TMC and 45 per cent. for CEX. There was little further excretion of any of the antibiotics between 6 hr and 24 hr.

Immediately after injection, 91 per cent. of the CER, 80 per cent. of the TMC and 73 per cent. of the CEX could be recovered from the gut (see figure).

**TABLE II**

Percentage of a 5-mg dose of cephalosporin present in the gastro-intestinal tract* and the blood†, and cumulative percentage excreted in the urine, at various times after intraduodenal injection into rats with unligated intestines

<table>
<thead>
<tr>
<th>Antibiotic‡; site of recovery</th>
<th>Percentage recoverable at hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CER Intestine . .</td>
<td>63·5</td>
</tr>
<tr>
<td>Serum . .</td>
<td>0·27</td>
</tr>
<tr>
<td>Urine . .</td>
<td>...</td>
</tr>
<tr>
<td>Total . .</td>
<td>63·77</td>
</tr>
<tr>
<td>TMC Intestine . .</td>
<td>60·0</td>
</tr>
<tr>
<td>Serum . .</td>
<td>0·5</td>
</tr>
<tr>
<td>Urine . .</td>
<td>0·06</td>
</tr>
<tr>
<td>Total . .</td>
<td>60·0</td>
</tr>
<tr>
<td>CEX Intestine . .</td>
<td>60·6</td>
</tr>
<tr>
<td>Serum . .</td>
<td>2·4</td>
</tr>
<tr>
<td>Urine . .</td>
<td>2·3</td>
</tr>
<tr>
<td>Total . .</td>
<td>63·0</td>
</tr>
</tbody>
</table>

* Total recovery from all four portions of the gastro-intestinal tract.
† Calculated on the assumption that the blood was 10 per cent. by weight of the body.
‡ CER = Cephaloridine; TMC = 7-(thienyl-2'-acetamido)-3-methylceph-3-em-4-carboxylic acid; CEX = cephalixin.

Within $\frac{1}{4}$ hr the total alimentary recovery of all three cephalosporins had fallen to about 60 per cent. of the initial dose and remained at that level until 1 hr after injection (see also table II). After this, the amounts of CER and TMC remaining in the gut fell slowly to reach 45 per cent. 6 hr after injection, but the amount of CEX fell rapidly, so that only 8 per cent. was present at this time.

The following observations were made on the distribution of antibiotic within the gut after intraduodenal injection. There was an immediate reflux into the stomach, amounting to some 30 per cent. of the antibiotic recoverable from the whole gut, and 1 hr later 2 per cent. of the CER, 15 per cent. of the TMC and 9 per cent. of the CEX remaining in the gut was still in the stomach. Two hours after injection the bulk of the antibiotic was in the small intestine, but by 4 hr CER and TMC had reached the caecum, and by 6 hr, 25 per cent.
of the CER, and 13 per cent. of the TMC was in the caecum and rectum; on the other hand, CEX was not present in the caecum and rectum in detectable amounts at any time.

When India ink was injected intraduodenally to act as a marker, some initial reflux into the stomach was observed. The dye reached the caecum in 4–6 hr.

Only 1 per cent. of the remaining CER and TMC was present in the duodenum after 6 hr, but 18 per cent. of all the CEX recovered from the gut at this time was in the duodenum.

**Effect of anaesthesia and laparotomy on the recovery of cephalaxin in the urine**

The shock of anaesthesia and laparotomy may affect intestinal motility and absorption from the gut may be different from that in normal animals (Small, Folen and Marcus, 1967). Urinary output and urinary recovery of CEX were therefore compared in normal and manipulated animals. After anaesthesia and laparotomy, urination was delayed for about 2 hr. The delay phase was seen in rats dosed both subcutaneously and orally. There was no accumulation of CEX in the urine in the 0–2 hr period after dosing such animals,
and it was inferred that both the production of urine and the excretion of cephalosporin were affected. The effects of anaesthetic and operative shock were probably general and not confined specifically to the alimentary tract.

**DISCUSSION**

When an antibiotic has been given orally, its presence in the blood and subsequent excretion in the urine is indisputable evidence that it has been absorbed from the gastro-intestinal tract. The relative concentrations of substances appearing in the blood after oral administration can be used to compare their absorption, provided the rate of removal of each from the blood is also known (Meyer and Lewis, 1963). However, if the blood concentration of the substances is the only information available, no conclusions can be drawn about the rate or site of their absorption from the gastro-intestinal tract.

If, after oral administration, the urine contains only a small amount of unchanged antibiotic but large amounts of degradation products, this may suggest that the substance has undergone decomposition in the intestine (Culp, Marshall and McMahon, 1963; Sullivan and McMahon, 1969), but the site or sites of degradation of the antibiotic cannot be identified.

When the three cephalosporins were given intraperitoneally to rats, they were found to have an almost identical serum half-life. Thus, comparison of the amounts present in the serum at any one time after oral dosing was a good measure of their relative rates of absorption. The accumulation of the three compounds in the urine over the 8 hr after dosing indicated similar relative rates of absorption for the compounds.

When the antibiotics were injected into ligated portions of the intestine, the serum levels obtained showed that CEX was absorbed poorly or not at all from the stomach and caecum, but was well absorbed throughout the length of the small intestine. CER and TMC were absorbed to only a small extent from all the sites tested.

The low absorption of all three substances from the stomach could not be attributed to destruction at that site, because substantial amounts could be recovered after incubating them with stomach homogenate for 4 hr (Ross et al., 1970). Again, after injection into the first 6 in. of the duodenum, minimal serum levels of CER and TMC were obtained, even though the good recovery of both substances from the gut showed that little had been destroyed. In contrast, although a similar proportion of the CEX injected was recovered from the gut, the serum concentration was six times greater than that of CER.

In the second part of the small intestine, poorer recoveries were obtained for all substances; however, whilst the recoveries of CER and CEX were the same, the serum levels of the two compounds indicated that nearly three times more CEX than CER was absorbed. This difference in absorption could not be attributed to the more rapid degradation of CER, because this did not occur (Ross et al.). The recovery of TMC was a little less than of CER and CEX, but the amount lost was not enough to account for its low serum concentration.
Much more loss of CER and of TMC was observed in the caecum than elsewhere. Even so, the serum concentration after injection of CER into the caecum was the same as that after it had been injected into the stomach, even though the apparent destruction in the stomach was very much less.

It was therefore concluded that the rat stomach does not absorb any of the three test substances and does not decompose them significantly. The duodenum and the small intestine, on the other hand, absorb CEX to a much greater extent than CER or TMC. Again, no rapid destruction of any cephalosporin was observed. This was in agreement with the in-vitro stabilities of the compounds to tissue homogenates and digestive enzymes of the small intestine (Ross et al.).

When the compounds were injected into the caecum, all three were poorly absorbed and CER and TMC were decomposed to a much greater extent, probably by the β-lactamases of micro-organisms present in this organ (Ross et al.). We conclude, therefore, that the region of maximal absorption is the duodenum and the rest of the small intestine, whilst the region of maximal destruction is the caecum. The reason why CEX gives higher blood levels than CER or TMC is that it is absorbed more rapidly than the other two substances; it is not due to the differing rates of degradation of the compounds, because CER and TMC were not destroyed before they could be absorbed. This conclusion was confirmed when the experiments were repeated with intraduodenal injection into unligated animals.

There was some evidence of biliary recycling of CEX; about 18 per cent. of the amount recovered from the whole alimentary tract was always found in the duodenum 6 hr after injection. A similar situation had been observed earlier with radioactively labelled cephaloram given intraperitoneally (Culp et al.). Constant recycling of this kind would enhance the absorption process. It was not observed with CER and TMC.

We cannot at present explain the initial fall in the recovery of the cephalosporins. They are not apparently decomposed by the digestive enzymes or by homogenates of the tissues in vitro (Ross et al.).

The differing absorption of the three cephalosporins probably reflects their differing chemical structures. The analogue with the 7-substituent derived from α-aminophenylacetic acid, CEX, was better absorbed than compounds in which the 7-substituent is derived from a substituted acetic acid without an α-substituent, such as CER or TMC. This result is consistent with those obtained by other authors, and suggests that the presence of the α-amino group in the 7-substituent makes a large contribution to the oral absorbability of CEX.

The nature of the substituent in the 3-position also influences the extent to which a cephalosporin is absorbed orally. A 3-substituent that is small and closely bound, such as the methyl group in CEX and TMC, appears to aid absorption, because TMC was better absorbed than was CER. Cephalexin is orally absorbed to a greater extent than is cephaloglycin, an analogue which also has phenylglycine in the 7-position, but acetoxy in the 3-position (Braun et al., 1968). The structure of cephalexin, combining a methyl group in the
3-position with phenylglycine in the 7-position may well show the maximum oral absorption possible with a cephalosporin compound.

**SUMMARY**

Cephalixin, cephaloridine and 7-(thienyl-2'-acetamido)-3-methylceph-3-em-4-carboxylic acid (TMC) were injected directly into the alimentary tract of rats and their subsequent fate was studied. The three antibiotics showed respectively good, poor and intermediate degrees of absorption from the alimentary tract. These differences are attributable to the rate at which each antibiotic is absorbed from the small intestine and not to the amount of degradation that occurs in the gut.

Cephalexin is much more rapidly absorbed from the small intestine than are cephaloridine and TMC. Cephalosporins that are not absorbed in the small intestine pass down the alimentary tract and are destroyed when they reach the caecum, from which there is little absorption.

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