Ampicillin Inactivation in the Caecum of Axenic, Gnotoxenic and Conventional Lambs: Interaction with Resistant or Sensitive Escherichia coli

By M. COSTE,1 PH. GOUET2* and L. ESCOULA1

1 Station de Pharmacologie-Toxicologie, INRA, Chemin de Tournefeuille, 31300 Toulouse, France
2 Laboratoire de Microbiologie, INRA Theix, 63122 Ceyrat, France

(Received 14 October 1983; revised 7 December 1983)

The fate of orally administered ampicillin was studied in axenic lambs, in gnotoxenic lambs given a complex microflora and a mixture of ampicillin resistant and/or sensitive strains of Escherichia coli, and in conventional lambs. In axenic lambs or animals with a sensitive microflora, antibiotic concentrations of 500-1600 µg ml⁻¹ were detected in the intestine, and most of the ampicillin passed through the small intestine and entered the large intestine, within 12-15 h of administration. These antibiotic concentrations were sufficient to decrease the numbers of ampicillin-sensitive E. coli from 10⁸-10⁹ bacteria ml⁻¹ to about 10⁵-10⁶ bacteria ml⁻¹ by 8 h after ampicillin administration. Second and third doses of antibiotic had no further effect on the bacterial count. Administration of ampicillin to animals hosting ampicillin-resistant E. coli resulted in a significant inactivation of the antibiotic in the intestine. As might be expected there was little reduction in the numbers of these organisms. These results are similar to those observed in conventional lambs hosting resistant E. coli as the dominant colibacillary flora.

INTRODUCTION

Ampicillin, a semi-synthetic antibiotic active against many Gram-positive and Gram-negative bacteria, is widely prescribed in veterinary medicine for treating pneumonia and septicaemia. In relation to plasma and serum concentrations the pharmacokinetics of ampicillin and of its derivatives are well understood (Smith & Hamilton-Miller, 1970; Tan et al., 1974; Ziv et al., 1977). Ampicillin is also given orally in neonatal diarrhoea therapy in the calf (Jones, 1974; Rolinson & Stevens, 1961). Studies on the fate of ampicillin in the digestive tract show considerable quantitative variations between individual animals of the same species (calves and pigs) (Acred et al., 1966; Escoula et al., 1982; Larkin, 1972). The presence of ampicillin-resistant (AmpR) organisms in the intestine, whether autochthonous or pathogenic, is likely to be a factor in these variations; both intracellular and extracellular β-lactamases produced by such organisms may be responsible for in situ degradation of this antibiotic in the digestive tract (Abraham & Chain, 1940; Bakhtiari & Selwyn, 1978; Lacey, 1980).

The present work attempted to study the overall effect of the digestive flora on the fate of ampicillin in conventional and axenic lambs, and the specific effect of sensitive or resistant Escherichia coli strains established either separately or in association with a reconstituted digestive flora in gnotoxenic lambs.

METHODS

Production and bacterial inoculation of axenic and gnotoxenic lambs. Axenic and gnotoxenic lambs were delivered by aseptic caesarian section then were immediately transferred to a sterile isolator according to the technique described by Riou et al. (1977) and Gouet et al. (1979). At 2 or 3 weeks of age, the lambs were fitted with a silastic cannula in the caecum, close to the ileo-caecal valve (Hecker, 1974; Pollock, 1964). They were bottle-fed twice daily.
Fig. 1. Inoculation steps of different bacterial strains and administration of ampicillin. Ax, axenic period. The arrows indicate the day of inoculation (CF, complex flora). The arrowheads indicate the oral administration of ampicillin. (1), (2) and (3) represent each inoculation sequence interval in the same animal; (a), (b) and (c) represent each inoculation sequence interval in another animal. □, *E. coli* AmpR; ▼, *E. coli* AmpS; ◼, complex flora.

Table 1. Numbers of animals and assays done

<table>
<thead>
<tr>
<th>Animal type</th>
<th>No. of animals</th>
<th>Total no. of antibiotic assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Axenic</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Gnotoxenic:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculation sequence (steps) in axenic lambs*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) <em>E. coli</em> AmpS</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>(2) <em>E. coli</em> AmpS + <em>E. coli</em> AmpR</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>(3) <em>E. coli</em> AmpS + <em>E. coli</em> AmpR + CF</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(a) CF</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>(b) CF + <em>E. coli</em> AmpS</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>(c) CF + <em>E. coli</em> AmpS + <em>E. coli</em> AmpR</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

* CF, complex flora (see text).

The gnotoxenic lambs were obtained by inoculating the axenic lambs *per os* on three consecutive days, with a complex flora (CF) of strains which had been isolated from the rumen and from the intestine of conventional lambs: *Lactobacillus acidophilus, Lactobacillus fermentum, Streptococcus faecalis* (a), *Streptococcus faecalis* (b), *Bifidobacterium* sp. (a), *Bifidobacterium* sp. (b), *Clostridium* sp., *Peptostreptococcus* sp., *Bacteroides ruminicola, Megasphaera elsdenii* and *Veillonella alcalescens*.

Two strains of *E. coli* were selected for their sensitivity or for their resistance to ampicillin, *E. coli* AmpS, for which the minimum inhibitory concentration (MIC) is 6 μg ml⁻¹ and *E. coli* AmpR, for which it is >1000 μg ml⁻¹. These strains were inoculated *per os* to axenic animals according to various steps (Table 1, Fig. 1), which made it possible to evaluate their effects on ampicillin metabolism. Thus in the same axenic lamb *E. coli* AmpS (step 1) then *E. coli* AmpR (step 2) and lastly, complex flora (CF) (step 3) were inoculated successively and separately. After each inoculation the flora was allowed to establish itself for 3 or 8 d.

Experiments began one week after the cannula was fitted. A dose of 120 to 170 mg water-soluble ampicillin was given orally (Penbritin Oral, Beecham) once a day at the morning feeding mixed with milk to obtain a dose rate of 17.5 mg per kg live weight.

Ampicillin concentration during passage through the caecum was determined from samples withdrawn two or three times from the caecal cannula. The sequence (1)→(2)→(3) was reproduced in two lambs and the sequence (2)→(3)→(1) in one lamb (Table 1, Fig. 1). A different sequence began with complex flora inoculation in two axenic lambs (step a) and three assays of antibiotic content were carried out. Then the *E. coli* AmpS strain (step b) was inoculated in the same animals as in step a, and five assays to determine antibiotic content were carried out. Lastly, in step c, *E. coli* AmpR was inoculated in one lamb only, and three assays were performed. Table 1 and Figure 1 show the steps, the number of animals and the number of assays performed. In addition to the gnotoxenic animals, two axenic and three conventional lambs were used (Table 1).
Ampicillin inactivation by E. coli in lambs

Enumeration of E. coli Amp<sup>R</sup> and Amp<sup>S</sup> and ampicillin dose. As soon as ampicillin was orally administered, 2 ml or 6 ml samples of caecal fluid were taken from the cannula every hour until the 15th hour.

Tenfold dilutions of the caecal contents were inoculated into a 1% (w/v) deoxycholate gelose agar (DCA, Institut Pasteur, Paris, France) to enumerate E. coli. Ampicillin-resistant bacteria were selected on DCA with ampicillin (100 μg ml<sup>-1</sup>) in the culture medium. Colonies were counted after 18 h incubation at 37 °C and the dominant colony-types in conventional animals were identified with a micro-method multitest system gallery (API 20E System SA; 38390 Montalieu Vercieu, France). The rest of the sample was centrifuged for 10 min at 15000 g and the supernatant was frozen at -25 °C.

Caecal fluid samples were assayed for ampicillin by a microbiological method (Arret et al., 1971) with Sarcina lutea (ATCC 9341). The standard curve was prepared in a solution containing one part phosphate buffer (0.1 M, pH 7.9) to one part caecal fluid without inhibitory action on Sarcina lutea.

RESULTS

Kinetics of ampicillin transit in the caecum

In axenic and conventional lambs, the lapse of time before the appearance of the maximum ampicillin concentration in the caecum was between 3 and 4 h after oral administration while it was from 5 to 8 h in gnotoxenic animals hosting E. coli Amp<sup>S</sup> either with or without the presence of the complex flora (Fig. 2).

In the three conventional experimental lambs, ampicillin was found in the caecum of only one animal, which yielded a maximal concentration of 230 μg antibiotic per ml caecal fluid (Fig. 2a). By contrast, in axenic lambs, the ampicillin concentration was more than 1500 μg ml<sup>-1</sup>. The complex flora, whether alone or in association with E. coli Amp<sup>S</sup>, had no significant influence on the amount of ampicillin in the caecum of gnotoxenic lambs (Fig. 2b, d). Similarly, in monoxenic animals harbouring only E. coli Amp<sup>S</sup>, the mean of the maxima obtained was always above 1000 μg ampicillin per ml caecal fluid. Conversely, in animals whose digestive tract was colonized by E. coli Amp<sup>R</sup>, the maximum antibiotic concentration observed did not exceed 245 μg ml<sup>-1</sup> (Fig. 2c, d).

At 24 h after ampicillin administration, a concentration of between 100 and 150 μg ml<sup>-1</sup> persisted in the caecum of all animals except the conventional and gnotoxenic lambs hosting E. coli Amp<sup>R</sup>, where ampicillin was no longer detectable. Lastly, it must be noted that there is a significant linear relationship (n = 14; r = 0.707 at P < 0.01) between the maximal concentration of ampicillin found in the caecum and the amount of milk consumed (Fig. 3).

Effect of ampicillin on E. coli

In the two conventional animals in which ampicillin was not detected in the digestive tract, the dominant caecal colibacillary flora was resistant to 100 μg ampicillin ml<sup>-1</sup>. In the gnotoxenic animals, whether or not the complex flora was present, the number of E. coli Amp<sup>S</sup> decreased 1000-fold in the caecum (from 10<sup>8</sup>-5 × 10<sup>9</sup> to 10<sup>5</sup>-10<sup>6</sup> bacteria ml<sup>-1</sup>) when antibiotic concentrations were maximal (i.e. between 490 and 1560 μg ml<sup>-1</sup>); the maximal decrease in the number of E. coli Amp<sup>S</sup> occurred between 6 h and 10 h after the antibiotic was administered, and generally occurred after the ampicillin peak (Fig. 4, bottom). The level of 10<sup>5</sup>-10<sup>6</sup> bacteria was maintained beyond the 24th hour as long as ampicillin concentrations greater than 60 μg ml<sup>-1</sup> were present in the caecum. When the antibiotic was administered a second time, the number of E. coli Amp<sup>S</sup> previously established was not affected by concentrations ≤1200 μg ml<sup>-1</sup>. Only once could a transitory drop be induced; this occurred at a maximum concentration of the antibiotic at 1600 μg per ml caecal fluid.

When the digestive tract harboured the Amp<sup>R</sup> strain, the first dose of antibiotic generally brought on a transitory drop in the number of E. coli Amp<sup>R</sup> (from 10<sup>8</sup>-10<sup>9</sup> to less than 4 × 10<sup>7</sup> bacteria) (Fig. 4, top). This drop was not repeated with the second and third treatment of the same animal. In one animal alone, the number of E. coli Amp<sup>R</sup> decreased to less than 10<sup>5</sup> ml<sup>-1</sup>. This animal initially harboured 10<sup>7</sup> E. coli when it received the antibiotic in a small amount of milk, which provoked the appearance of an ampicillin peak exceeding 1900 μg ml<sup>-1</sup>.
Fig. 2. Concentration of ampicillin in caecal fluid after oral administration. The points represent sample means ± SD for three (a, b) or five (c, d) assays. (a) •, Axenic; △, conventional. (b) ●, Gnotoxenic (complex flora). (c) ●, Gnotoxenic (E. coli AmpS); △, gnotoxenic (E coli AmpS + E. coli AmpR). (d) ●, Gnotoxenic (E. coli AmpS + complex flora); △, gnotoxenic (E. coli AmpS + E. coli AmpR + complex flora).

Fig. 3. Correlation of maximal concentration of ampicillin in the caecum and volume of milk ingested during antibiotic administration (axenic and gnotoxenic lambs without E. coli AmpR). $y = 1.025x + 1625; r = 0.707; n = 14.$
Ampicillin inactivation by *E. coli* in lambs

The presence of high concentrations of ampicillin in the caecum probably results from a concentration mechanism in the anterior gastrointestinal tract, which has been described for other antibiotics (Mylrea, 1966, 1968).

The kinetics determined in gnotoxenic animals strongly suggest the involvement of *E. coli* AmpR in ampicillin inactivation in the digestive tract. Although these results do not exclude the possibility that other bacteria metabolize ampicillin (Pollock, 1964), they are in agreement with the previously observed relationship between the level of the resistant colibacillary flora and the fate of this antibiotic in the digestive tract of the conventional pig (Escoula *et al.*, 1982). Even relatively low concentrations of ampicillin are sufficient to reduce the number of AmpR *E. coli* to $10^5$ ml$^{-1}$. The latter population is not, however, modified even with concentrations up to 1200 μg ml$^{-1}$. This is probably linked to the presence of ecological niches inaccessible to the antibiotic and/or the reversible transformation of *E. coli* into filamentous forms that become insensitive to the antibiotic (Rolinson *et al.*, 1977). This threshold effect was also noted in the caecal colibacillary flora of conventional pigs (Escoula *et al.*, 1982). It should be noted that there was no correlation between the sensitivity (MIC) of *E. coli* determined *in vitro* and results obtained *in vivo*.

We have observed that ampicillin reduces the number of *E. coli* AmpR only during its first administration. This finding could be explained by the fact that extracellular β-lactamase is inducible (Lacey, 1980), requiring contact between the organism and the antibiotic; during the second and third administrations of ampicillin the population of *E. coli* AmpR may be fully induced and hence less sensitive to the antibiotic.

This study demonstrates the potential importance of a β-lactamase-resistant colibacillary flora within the digestive microflora, but other bacterial strains also play a critical role in the fate of certain antibiotics (Kikuchi *et al.*, 1973). Therapeutically, the efficacy of the β-lactam antibiotics depends as much on the sensitivity of commensal colibacillary flora as on the sensitivity of the pathogen involved. The autochthonous flora of the digestive tract can in fact be considered in some cases to be an 'indirect factor of pathogenicity' (Maddocks & May, 1969).
Ampicillin and colistin are often prescribed together in veterinary medicine because of their synergistic action observed in vitro. However, this action should be verified in vivo because colistin can select bacteria capable of inactivating ampicillin (Proteus spp.) (Coste & Escoula, 1983). Similarly lysis of E. coli Ampβ by colistin could lead to the release of a greater pool of β-lactamase in the digestive tract.

We wish to thank M. Chavarot, G. Andant, G. Vert and Josette Gouet for their valuable technical assistance.

REFERENCES


