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Existence of a specialized absorption mechanism for cefadroxil, an aminocephalosporin antibiotic, in the human oral cavity

Yuji Kurosaki, Hidekatsu Nishimura, Kazuyuki Terao, Taiji Nakayama and Toshikiro Kimura

Faculty of Pharmaceutical Sciences, Okayama University, Okayama (Japan)

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Summary

The absorption of cefadroxil, an aminocephalosporin antibiotic, from the oral cavity of healthy volunteers was examined. The buccal absorption test for cefadroxil at varying concentrations demonstrated the phenomenon of saturation in absorption behavior. Furthermore, absorption of cefadroxil was inhibited by the presence of another aminocephalosporin, cephalexin, but not by the structurally unrelated sulfisoxazole. The results suggest that aminocephalosporin antibiotics are absorbed from the human oral cavity via a specialized transport mechanism. On the other hand, cefadroxil absorption from hamster cheek pouch mucosa, a keratinized oral mucosa, took place in proportion to the initial concentration and was not influenced by the presence of cephalexin, indicating that the physicochemical interaction between these two aminocephalosporins in the luminal solution of the keratinized oral mucosa is practically negligible. Thus, the localization of the specialized absorption mechanism for cefadroxil was strongly suggested to be in the non-keratinized region in the human oral cavity.

Introduction

The oral mucosa is an attractive site for drug administration in order to improve the bioavailability of drugs susceptible to extensive presystemic elimination, since drugs absorbed from the oral mucosa can thereby avoid elimination via both intra-alimentary canal and hepatic first-pass routes (Bell et al., 1985; Hussain et al., 1987; Kurosaki et al., 1988). For investigations on drug absorption from the human oral cavity, the buccal absorption test established by Beckett and Triggs (1967) has generally been used. The passage of most acids and bases across the human oral mucosa can be explained based on the mechanism of passive diffusion of unionized molecules (Beckett and Moffat, 1969, 1970; Bickel and Weder, 1969). We also confirmed by using hamster cheek pouch mucosa that drug absorption across the keratinized oral-mucosal membrane takes place due to a passive diffusion mechanism and behaves according to the pH-partition hypothesis (Kurosaki et al., 1986, 1987). On the other hand, it has recently been reported that absorption of a number of nutrients such as nicotinic acid, nicoti-

Correspondence: T. Kimura, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka 1-1-1, Okayama 700, Japan.

namide (Evered et al., 1980), thiamine (Evered and Mallet, 1983) and glutathione (Hunjan and Evered, 1985) from the human oral cavity may occur via carrier-mediated processes. However, regionally different oral mucosae exhibit marked histological variation, such as the degree of keratinization or the thickness of the epithelia (Berkovitz et al., 1978), and therefore characterization of the oral mucosae was thus far incomplete in terms of regional differences in drug absorption (Yamahara et al., 1990; Kurosaki et al., 1991).

Amino-β-lactam antibiotics are well known to undergo absorption from the small intestine via the carrier system for dipeptide transport (Kimura et al., 1978, 1983, 1985; Umeniwa et al., 1979; Nakashima et al., 1984; Okano et al., 1986). Cefadroxil, an aminocephalosporin antibiotic (Fig. 1a), is one example for which we have demonstrated the presence of a carrier-mediated transport system in rat small intestine (Kimura et al., 1983, 1985).

In the present study, the mechanism of absorption of cefadroxil from the oral cavity was examined and the localization of the specialized transport system for cefadroxil in human oral mucosa was determined via comparative examination in hamster cheek pouch mucosa.

Materials and Methods

Chemicals

Cefadroxil (Bristol Meyers Co., Tokyo) and cephalexin (Fujisawa Pharmaceutical Co., Osaka) were used as supplied. All other reagents were reagent grade commercial products and were used without further purification.

Preparation of drug solution

Drugs were dissolved in an isotonic buffer solution of NaH_2PO_4-Na_2HPO_4, pH 6.5.

Procedure for in vivo absorption experiments in human oral cavity

Buccal absorption experiments were carried out according to the method of Beckett and Triggs (1967). Seven healthy volunteers, six males and one female, aged 21–34 years, participated in the study. Cefadroxil solution (10 ml) containing an unabsorbable marker, phenol red (15 μg/ml), was placed in the mouth. Phenol red was added as a marker to correct for drug loss on swallowing. The drug solution in the mouth was vigorously agitated using the cheeks and tongue for 5 min, and then the solution was expelled as completely as possible. The subject quickly rinsed his/her mouth three times with the same buffer solution (total volume, 20 ml), and expelled the rinsings. The expelled solutions were combined and were adjusted to 50 ml with the buffer solution. From the values of the initial and final concentrations of the drug (C_{di} and C_{df}, respectively) and the marker (C_{mi} and C_{mf}, respectively), the percentage of drug absorbed during a period of 5 min was calculated as follows:

$$\text{Absorption } (\%) = \{1 - (C_{df}/C_{di}) \times (C_{mi}/C_{mf})\} \times 100.$$

Procedure for in vivo absorption experiments in hamster cheek pouch

Male golden hamsters (110–160 g body weight) were anesthetized with urethane (1.5 g/kg, i.p.) and were fastened onto the previously described platform (Kurosaki et al., 1986). The cheek pouch was cleaned by multiple rinses with buffer solution and the mucosal surface was wiped with cotton balls to remove excess water. 2 ml of drug solution were administered into the cheek pouch. After 1 h, the luminal contents were withdrawn and the cheek pouch was washed with the buffer solution. The washings were combined with the luminal contents and the total volume was adjusted to 20 ml. The difference in the amounts of drug between the initial and final solutions was defined as the amount absorbed.

Analytical methods

Cefadroxil concentration was determined by HPLC. Samples were filtered through a 0.45 μm pore size membrane filter (Nihon Millipore Kogyo Co., Ltd, Yonezawa, Japan). An appropriate volume of the filtrate was injected into the liquid

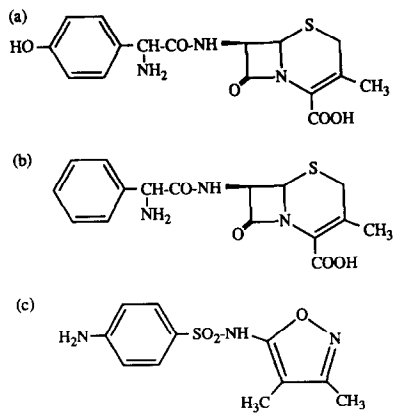

Fig. 1. Chemical structures of (a) cefadroxil, (b) cephalexin, and (c) sulfisoxazole.

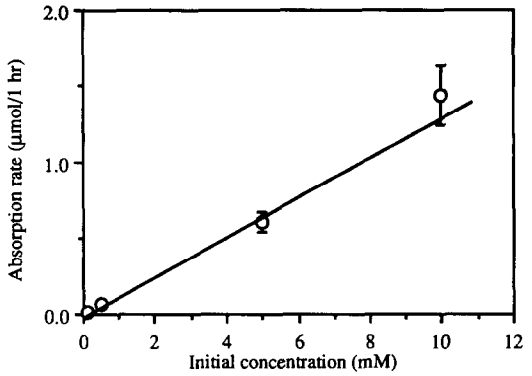

Fig. 3. Rate of absorption of cefadroxil from hamster cheek pouch. Hamster cheek pouch method of Kurosaki et al. (1986) was carried out for 1 h using 2 ml of cefadroxil solution (pH 6.5). Results are expressed as means ± S.E. of 4–7 experiments.

chromatograph. The HPLC system consisted of a pump (LC-5A; Shimadzu, Kyoto), a column (4.6 mm i.d. × 150 mm) packed with 5 μm Inertsil ODS (Gasukuro Kogyo, Tokyo) and a spectrophotometric detector (SPD-2A; Shimadzu). The wavelength of the detector was set at 262 nm. The degassed mobile phase consisted of 0.01 M ammonium acetate-methanol (93 : 7 v/v) at a flow rate of 1.2 ml/min. The drug concentration was calculated from a calibration line constructed on the basis of peak-area measurements.

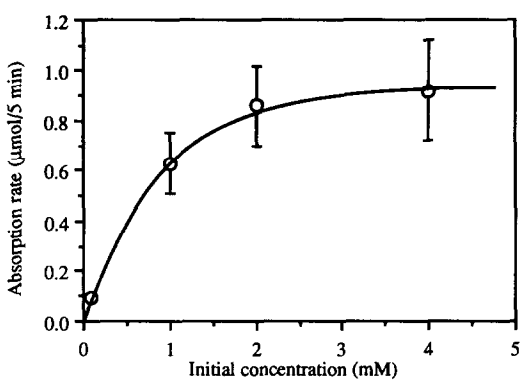

Fig. 2. Rate of absorption of cefadroxil from human oral cavity. Buccal absorption test of Beckett and Triggs (1967) was carried out for 5 min using 10 ml of cefadroxil solution (pH 6.5). Phenol red (15 μg/ml) was added to the solution to correct for drug loss due to swallowing during the test. Results are expressed as means ± S.E. of 4–7 volunteers.

Results

The concentration dependency of cefadroxil absorption from both the human oral cavity and hamster cheek pouch was examined and the results are shown in Figs 2 and 3, respectively. As is evident from Fig. 2, apparent saturation was observed in cefadroxil absorption from the human oral cavity. On the other hand, the percentage absorption of cefadroxil from hamster cheek

TABLE 1

Effect of cephalexin and sulfisoxazole on oral mucosal absorption of cefadroxil in human

Additive	Cefadroxil absorption [a] (% absorbed in 5 min)
None	9.2 ± 1.4
Cephalexin [b]	4.2 ± 1.1 [c]
Sulfisoxazole [b]	9.2 ± 1.6 [d]

Results are expressed as means ± S.E. of seven volunteers.
[a] Initial concentration of cefadroxil: 0.1 mM.
[b] Initial concentration of additive: 1.0 mM.
[c] Significantly different from None ($p < 0.01$) by paired t-test.
[d] Not significantly different from None ($p > 0.05$) by paired t-test.

TABLE 2

Effect of cephalexin on mucosal absorption of cefadroxil from hamster cheek pouch

Additive	Cefadroxil absorption [a] (% absorbed in 1 h)
None	6.8 ± 0.8 (7)
Cephalexin [b]	7.3 ± 0.9 [c] (11)

Results are expressed as means ± S.E. with the number of experiments in parentheses.

[a] Initial concentration of cefadroxil: 0.1 mM.

[b] Initial concentration of cephalexin: 1.0 mM.

[c] Not significantly different from None ($p > 0.05$) by Student's *t*-test.

pouch over a 1 h period was found to be independent on the initial concentration; namely, the rate of absorption was proportional to the initial concentration studied (Fig. 3).

Table 1 lists the effects of cephalexin (Fig. 1b) and sulfisoxazole (Fig. 1c) on buccal absorption of cefadroxil in human volunteers. Absorption of cefadroxil (0.1 mM) was markedly inhibited by the presence of the structurally similar cephalexin (1.0 mM), while no influence was found in the case of the structurally unrelated sulfisoxazole (1.0 mM). On the other hand, the absorption of cefadroxil (0.1 mM) from hamster cheek pouch, a keratinized oral mucosa, was not inhibited by the presence of 1.0 mM cephalexin (Table 2).

Discussion

We have demonstrated that cefadroxil is absorbed from rat small intestine through a carrier-mediated process and that cefadroxil (0.1 mM) absorption in rat small intestine was markedly inhibited by the presence of 1.0 mM cephalexin (Kimura et al., 1983). As shown in Fig. 2, the rate of absorption of cefadroxil from the human oral cavity seems to be saturable or of limited capacity. In addition, cefadroxil absorption was inhibited by cephalexin, which is absorbed from rat small intestine via a common carrier-mediated process with cefadroxil, but not by sulfisoxazole, which is absorbed according to a mechanism of passive diffusion (Table 1). These results suggest the possibility that cefadroxil absorption from the human oral cavity takes place through a carrier-mediated mechanism which is common to cephalexin.

The existence of specialized transport systems for some nutrients, i.e., nicotinic acid, nicotinamide (Evered et al., 1980), thiamine (Evered and Mallet, 1983) and glutathione (Hunjan and Evered, 1985), in the human oral cavity has been reported using the human buccal absorption test of Beckett and Triggs (1967). Unfortunately, no information on the regional differences in such a specialized process of absorption can be obtained from the results of the human buccal absorption test. Regionally different oral mucosae exhibit evident variations in morphology (Squier and Hall, 1985). Regional differences in drug absorption from oral mucosae have been reported in dogs (Yamahara et al., 1990) and hamsters (Kurosaki et al., 1991). In the latter report, we discussed the regional permeability of salicylic acid in hamster oral mucosa along with morphological variations, especially in the degree of keratinization (Kurosaki et al., 1991). However, the oral mucosae have not yet been characterized sufficiently in terms of regional differences in drug absorption including the existence or localization of specialized transport systems. Human oral mucosa can be classified into two groups; keratinized and non-keratinized mucosa. Lip, attached gingiva, the dorsum of the tongue and hard palate have keratinized mucosa, whilst buccal and sublingual mucosa and the ventral surface of the tongue are not keratinized (Berkovitz et al., 1978).

In this study, two different model systems were used to make assumptions about the location of the non-linear transport system for cefadroxil. One was the human buccal absorption test (Beckett and Triggs, 1967) in which drug molecules might be absorbed from the entire surface of the oral cavity. The other was based on the hamster cheek pouch method (Kurosaki et al., 1986). The absorption experiment under conditions similar to those of the human buccal absorption test was carried out in hamster cheek pouch which was used as a model for keratinized oral mucosa (Kurosaki et al., 1986, 1987). The amount of cefadroxil absorbed from the cheek pouch was

found to be directly proportional to the initial concentration (Fig. 3) and the absorption process was not inhibited by cephalexin (Table 2). The results suggest that the two drugs, cefadroxil and cephalexin, do not undergo physicochemical interaction with each other in the luminal solution and that the specialized transport process appears to exist in the non-keratinized regions of human oral mucosae.

The present article is the first report demonstrating the existence of a specialized transport system for cefadroxil, a xenobiotic, in human oral mucosa, the probable location of the system being within the non-keratinized region of the oral cavity. However, the exact location of this specialized transport system and the characteristics of the specialized drug absorption system remain to be clarified.

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