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Intestinal Absorption Aspect of Non-lipophilic Low Molecular Weight Drugs: A Case of Cephalexin and Cefazolin

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Absorption characteristics of cephalosporins were investigated using *in situ* recirculation technique. Cephalexin as a fairly well absorbed cephalosporin, and cefazolin as a poorly absorbed one were selected as model compounds. Absorption of cephalexin was much faster in its isoelectric region than in alkaline pH. The pH-absorption profile was not consistent with the pH-partition behavior. The absorption of cephalexin was as much as 4.6 times of cefazolin. This difference could not be explained by the pH-partition hypothesis. The surface activities of cephalosporins were investigated and their contributions to the absorption characteristics were little. The transfer rate of cephalosporins from aqueous lecithin liposome dispersion was investigated. The membrane transfer rate of cephalexin was markedly faster than cefazolin and similar result was obtained when liposome was prepared from rat intestinal total lipids. The pH-profile of the transfer rate of cephalexin across the lipid bilayers was similar to the pH-absorption profile. It is suggested that liposomes prepared from total lipids of the intestine as well as lecithin liposomes are suitable and efficient models for investigation of the transfer of these drug molecules across the intestinal membrane.

Keywords——intestinal absorption; cephalexin; cefazolin; *in situ* recirculation; lecithin-liposome; total lipids-liposome; surface tension; pH-partition hypothesis; rat

Cephalosporin is considered one of the most important antibiotics. Production of 7-amino cephalosporanic acid from cephalosporin C facilitated the production of a large number of derivatives with varying physical, biological and antimicrobial properties.

Semisynthetic cephalosporins can be divided into two classes on the basis of their absorption characteristics, those which can be absorbed from the gastrointestinal tract as cephaloglycine, cephalexin and cephradine, and the others which are poorly absorbed as cephalothin, cephaloridine and cefazolin.

In spite of their therapeutic importance and wide applications, the absorption mechanisms and the factors determining their absorption characteristics have not been established. The present investigation was undertaken to clarify the differences in absorption characteristics of cephalosporins. Cephalexin and cefazolin were selected as model compounds.

Experimental

Materials——Cephalexin monohydrate was a gift from Takeda Chemical Industries, Ltd., Osaka, Japan. Sodium cefazolin·5H_2O and [7-^{14}C] sodium cefazolin (1.5 μCi/mg) were a gift from Fujisawa Pharmaceutical Industry Ltd., Osaka, Japan. Phosphatidylcholine was prepared from egg yolks according to the method of Rhodes and Lea.[2] Rat intestinal mucosa was scraped off with a cover glass and the total lipids were extracted as described by Folch and others.[3] All other chemicals and solvents were reagents grade unless otherwise indicated.

Buffer's Composition——The isotonic buffer solutions of pH 5.3 and 6.5 were prepared from 0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4 and that of pH 8.3 was prepared from 0.165 M $NaHCO_3$. Buffers for the partitioning experiments were prepared with 0.2 M Na_2HPO_4 and 0.1 M citric acid.

1) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto.*
2) D.N. Rhodes and C.H. Lea, *Biochem. J.*, **65**, 526 (1957).
3) J. Folch, M. Lees, and G.H. Sloane Stanley, *J. Biol. Chem.*, **226**, 497 (1957).

Absorption Studies——Male Wistar albino rats 160—210 g were used. *In situ* recirculation technique of Kakemi and others[4] was employed. The bile duct was ligated in all experiments. All the absorption experiments were carried out at 37°. The perfusion solution (40 ml) was recirculated at a rate of 5 ml min^{-1}. At the end of the specified period, the perfused solution was withdrawn and the intestine washed with pH 6.5 buffer solution. The perfused solution and washings were combined together and completed to 100 ml with the same buffer. Then, the residual amount of drug was determined and the amount disappeared was calculated by difference.

Apparent Partition Coefficient Measurements——These were done by the method of Suzuki and others.[5]

Surface Tension Measurements——These were done at 25° using a Du Noüy Tensiometer (Shimadzu).

Preparation of Liposomes——Two kinds of liposomes were prepared, one from phosphatidylcholine (40 μmole) and dicetylphosphate (10 μmole), and the other from total lipids of the intestine (45 mg) according to the method of Kinsky and others.[6]

Transfer Rate Measurements——The overall transfer rate of cephalosporins from aqueous liposome dispersions was determined by the method of dynamic dialysis of Meyer and Guttman,[7] except that the internal solution in the Visking Cellulose Tube (20/32) was 6 ml, and the external solution was 60 ml. Temperature was maintained at 37°.

Analytical Methods——Cephalexin and cefazolin were determined spectrophotometrically according to the method of Purich and others[8] with the exception that cephalexin was measured at 261 mμ and cefazolin at 271 mμ.

Cephalexin in Perfusion Solution: One ml of 2 N sodium hydroxide was added to 3 ml sample, the mixture was heated at 98±2° for 30 min, a strong fluorescence was formed, which was reported in a recent publication by Yamana and others,[9] and its intensity was measured at an excitation maximum of 355 nm and an emission maximum of 433 nm.

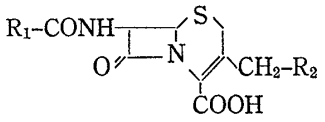
7-^{14}C-Sodium Cefazolin in Perfusion Solution: This was determined by measuring its radioactivity with a liquid scintillation counter (Beckman LS-232).

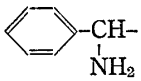
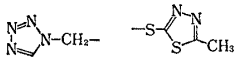
D-Glucose was determined enzymatically using the Glucose UV Test (Boehringer-Mannheim).

Results and Discussion

The structural formulas of cephalexin and cefazolin are presented in Table I. Both have the same central nucleus, 7-amino cephalosporanic acid. Cefazolin is a monobasic cephalo-

Table I. Structures and Physico-chemical Properties of Cephalosporins



	R_1	R_2	pK_a	Apparent partition coefficient (*n*-BuOH/buffer, 37°) pH 5.3	6.5	8.0
Cephalexin		H	5.2, 7.3[a]	0.20	0.35	0.95
Cefazolin			2.3[b]	0.39	0.38	0.59

a) C.W. Ryan, *J. Med. Chem.*, **12**, 310 (1969)
b) C.H. Nightingale, *J. Pharm. Sci.*, **64**, 1899 (1975)

4) K. Kakemi, T. Arita, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).
5) E. Suzuki, M. Tsukigi, S. Muranishi, H. Sezaki, and K. Kakemi, *J. Pharm. Pharmacol.*, **24**, 138 (1972).
6) S.C. Kinsky, J. Haxby, C.B. Kinsky, R.A. Demel, and L.L.M. Van Deenen, *Biochim. Biophys. Acta*, **152**, 174 (1968).
7) M.C. Meyer and D.E. Guttman, *J. Pharm. Sci.*, **59**, 33 (1970).
8) E.D. Purich, J.L. Colaizzi, and R.I. Poust, *J. Pharm. Sci.*, **62**, 545 (1973).
9) T. Yamana, A. Tsuji, K. Kanayama, and O. Nakano, *J. Antibiotics* (Tokyo), **27**, 1000 (1974).

sporin, whereas cephalexin is an amphoteric one and has three ionic species (cation, zwitterion and anion). The percent of total concentration of each ionic species has been calculated as a function of pH by Purich and others.[8] Within the isoelectric range cephalexin exists essentially as an electrically neutral zwitterion, whereas, at lower or higher pH's it exists mainly as a cation or an anion respectively.

In order to examine the differences in their lipid solubility we measured the apparent partition coefficient at different pH's, using *n*-butanol as an organic phase. It is clear in Table I that in case of cephalexin it was increased on increasing pH, which is in agreement with that reported by Purich and others.[8]

In a recent publication Purich[10] illustrated that the rate of transfer of cephalexin across the everted rat intestine increased as the mucosal solution was made more alkaline. Therefore, we tried to investigate the influence of pH on the intestinal absorption of cephalexin *in situ*. As it is shown in Fig. 1, the percentage absorption of cephalexin at pH 8.3 was 5.4±2.0%, in contrast to its absorption from the other solutions of different pH's within its isoelectric range, where it was fairly well absorbed. There is a discrepancy between our results and those reported by Purich,[10] which could be attributed to a difference in the experimental conditions, and this was previously demonstrated by Perrier and Gibaldi.[11] They have demonstrated that the everted gut clearance data for two cephalosporins (cephalexin and cephaloridine) were not in agreement with the *in situ* absorption results, and stated that the everted gut technique is unsuitable as a model for predicting human drug absorption in case of cephalosporins.

At the same time, no correlation between the apparent partition coefficient (TableI) and pH-absorption profile of cephalexin (Fig. 1) could be demonstrated. Consequently, it seems to be difficult to explain the absorption mechanism of cephalexin on the basis of the pH-partition hypothesis developed by Schanker and others.[12] Moreover, the apparent partition coefficient of cefazolin at pH 6.5 was slightly greater than that of cephalexin. According to the

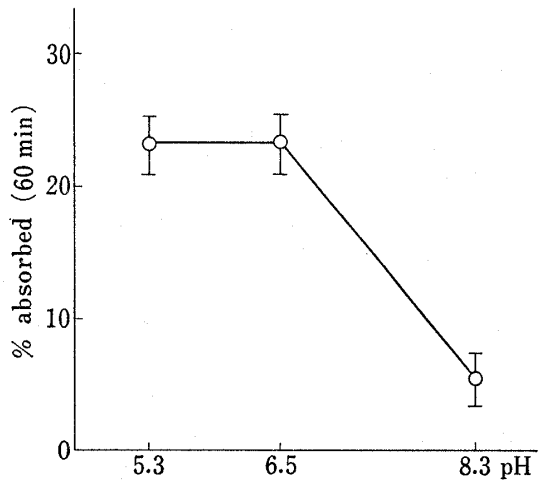


Fig. 1. Effect of pH on the Intestinal Absorption of Cephalexin *in Situ*

Cephalexin (0.1 mM) was dissolved in isotonic buffer at various pH values and perfused through the rat small intestine for one hour. Vertical bars represent the mean ± S.D. of at least 4 rats.

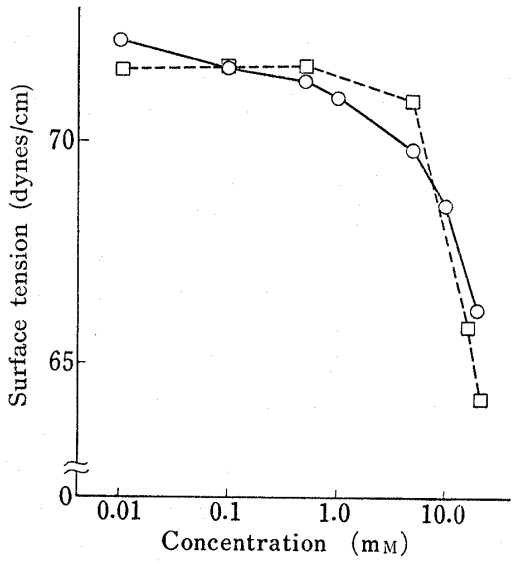


Fig. 2. Surface Tension of Cephalosporins at Various Concentrations

Drugs were dissolved in pH 6.5 phosphate buffer and the surface tension was measured at 25°, using a Du Noüy Tensiometer. ○: cephalexin, □: cefazolin
surface tension of pH 6.5 phosphate buffer: 72.78 dynes/cm

10) E.D. Purich, *Diss. Abstr. Int. B*, **34**, 2122 (1973).
11) D. Perrier and M. Gibaldi, *J. Pharm. Sci.*, **59**, 33 (1973).
12) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).

pH-partition hypothesis, cefazolin should be absorbed as much as cephalexin at the same pH. But, on the contrary, the percentage absorption of cefazolin at pH 6.5 at the end of 1 hr was only 5.0±1.1%. Thus, it is evident that the absorption of cephalosporins is not in accordance with the pH-partition hypothesis.

Previous observations of Quay[13] indicated the presence of an active transport mechanism of cephalexin and also demonstrated a competition between cephalexin, glycine and L-phenylalanylglycine during their absorption from the rat jejunum. On the contrary, Penzotti and Poole[14] have recently shown that the β-lactam antibiotics are not transported across the everted rat gut by any specialized transport mechanism. Consequently, our researches were extended to illustrate the mechanism of transport of cephalexin using *in situ* recirculation technique. This was done by investigating the effect of increasing the concentration of cephalexin on its intestinal absorption, and also by the addition of other compounds such as amino acids or dipeptides to demonstrate whether they can compete with it and affect its absorption. It was found that the percentage absorption of cephalexin at the end of 1 hr using various concentration of 0.01, 0.1, 1.0, and 10 mM were 25.7±3.9, 23.2±2.3, 21.3±4.4, and 23.2±5.6% respectively, which shows no significant difference in the absorption of cephalexin and indicates that its absorption was not saturable over a 1000 fold change in concentration. At the same time, the absorption of cephalexin (0.01 mM) was not affected by the presence of L-phenylalanylglycine (1 mM). This demonstrates that the competition between the two compounds during the absorption process was not occurred. Hence, these studies could not provide any evidence for active transport of cephalexin.

It can be rationalized that the surface activity of the various dissociative species of drugs may be able to influence their absorption characteristics. Purich[10] has shown a linear correlation between the change in *in vitro* transport rates of cephalexin and the surface excess which was calculated from the surface tension reduction of cephalexin buffer solutions. Therefore, surface tension measurements of cephalosporins at various concentrations were made. Figure 2 illustrates no significant difference between the surface tension of cephalexin and cefazolin at lower and higher concentrations, when they were measured at pH 6.5, although their surface tension was reduced at higher one. Moreover, the surface tension of cephalexin was also measured at pH 5.3 and 8.3, and it was found that the extent of the surface tension reduction of cephalexin was the same within the concentration range of 0.01—10 mM, at which absorption experiments were carried out. From these data, it has been suggested that surface activity of the cephalosporins contributes little to their absorption characteristics.

As it has been known that the passage of drug molecules through the plasma membrane of the intestinal epithelium constitutes the first stage in drug absorption, it is worthy to investigate the membrane transport characteristics of cephalosporins in order to clarify the difference in absorption between cephalexin and cefazolin. Therefore, the membrane transfer rate was investigated using lecithin liposome dispersions as model membrane. As it is obvious in Fig. 3, the rate of transfer of free cephalexin or cefazolin across the Visking dialysis sac was significantly rapid, therefore their transport across the lipid bilayers of liposomes could be taken as the rate limiting process of their passage through the bimolecular layer and diffusion to the external surrounding medium. It is evident in Fig. 3, that the rate of cefazolin transfer across the membrane was markedly slower than cephalexin, which is in consistent with our previous observations concerned with their intestinal absorption. This result contributes to a certain extent in explaining the reason of intestinal absorption characteristics between cephalexin and cefazolin.

Then we examined the effect of pH on the rate of transfer from lecithin liposome dispersions. But, since the salts used for preparation of buffers at pH 8.3 and that at pH 5.3 and

13) J.F. Quay, *Physiologist*, **13**, 287 (1970); *idem*, *ibid.*, **15**, 241 (1972).
14) S.C. Penzotti Jr. and J.W. Poole, *J. Pharm. Sci.*, **63**, 1803 (1974).

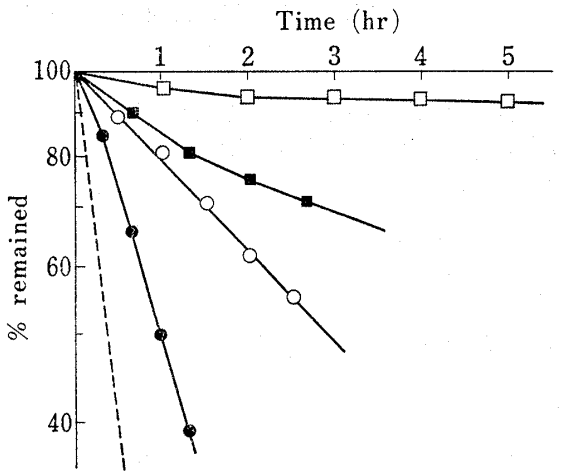


Fig. 3. Overall Transfer Rate of Cephalosporins from Aqueous Liposome Dispersions Prepared from Egg Lecithin or Small Intestinal Total Lipids (pH 6.5, 37°)

------: free cephalexin or cefazolin
—○—: cephalexin-egg lecithin liposome
—□—: cefazolin-egg lecithin liposome
—●—: cephalexin-total lipid liposome
—■—: cefazolin-total lipid liposome

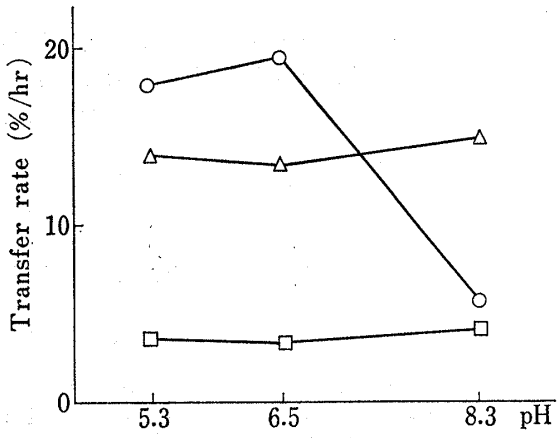


Fig. 4. Effect of Bulk pH on the Overall Transfer Rate of Cephalosporins and Glucose from Aqueous Egg Lecithin Liposome Dispersions

—○—: cephalexin, —□—: cefazolin, —△—: glucose

6.5 were different, control experiments using glucose as a marker were carried out to examine the influence of the compositional change of buffers on the permeability of liposomes. As it is shown in Fig. 4, the transfer rate of glucose from lecithin liposome dispersions remained constant irrespective of the change in pH and buffer constituents. This shows that the influence of buffer composition on the rate of transfer of drugs from liposomes is negligible.

From Fig. 4, it can be seen that the rate of transfer of cephalexin was markedly decreased at pH 8.3, while that of cefazolin was not affected. Furthermore, a good correlation between the apparent pattern of the pH-transfer rate profile of cephalexin (Fig. 4) and that of the pH-absorption profile (Fig. 1) could be noticed. Therefore, it is conceivable to ascribe the decrease in absorption of cephalexin at pH 8.3 to a decrease in the membrane transfer rate. The transport across the lipid bilayer can be considered as the rate limiting process of the intestinal absorption of cephalexin.

Lecithin liposomes used in the present study as model membrane were consisted of phosphatidylcholine and dicetylphosphate. Whereas, the plasma membrane of intestinal epithelium consists of various kinds of lipids, these are cholesterol, free fatty acid, phospholipids and glycolipids. Consequently, as an approach to what occurring at the natural plasma membrane during the absorption processes, we intended to prepare liposomes from the total lipids, which were extracted from the rat small intestinal mucosa by the method of Folch and others.[3] That is because if the constituents of the model membrane could be made corresponding to the lipid constituents of the plasma membrane, more valuable knowledge concerning with the transfer of drug molecules across the natural membranes can be obtained. From Fig. 3, it is clear that the rate of transfer of cephalexin across the liposomal membrane prepared from total lipids was faster than that of cafazolin. This is in agreement with the results obtained from the lecithin liposome dispersions and is considered an additional evidence confirm the existence of a difference in membrane transfer rate between cephalexin and cefazolin. On the other hand, these results show that liposomes prepared from total lipids of the intestine as well as lecithin liposomes are suitable and efficient models for investigation of the transfer of these drug molecules across the intestinal membrane.