Possible Mechanism Behind the Adjuvant Action of Phosphate Derivatives on Rectal Absorption of Cefoxitin in Rats and Dogs

TOSHIAKI NISHIHATA ˣ, **CHIA-SHUN LEE**, **BA THU NGHIEM**, and **TAKERU HIGUCHI**

Received July 18, 1983, from the *Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045.* Accepted for publication January 19, 1984.

Abstract □ The mechanism underlying the ability of phosphate derivatives to act as adjuvants and coadjuvants was examined in *in vivo* and *in situ* preparations in rats and dogs. The adjuvant effect of DL-α-glycerophosphate on the rectal absorption of sodium cefoxitin was greatly augmented by the presence of either sodium phytate or sodium tripolyphosphate. These coadjuvants only slightly enhanced cefoxitin rectal absorption when administered alone. Therefore, enhancement of the permeation of tripolyphosphate and phytate by α-glycerophosphate may be necessary before the coadjuvants can significantly affect cefoxitin absorption. The inhibitory effect of disodium 4,4′-diisothiocyano-2,2′-disulfonate stilbene, which is known to interact with the amino group in the protein fraction, on enhanced cefoxitin absorption suggests the involvement of the protein fraction in transport of cefoxitin across the rectal membrane.

Keyphrases □ Absorption—rectal, cefoxitin, rats and dogs □ Phosphate derivatives—adjuvant or coadjuvant action □ Adjuvants—α-glycerophosphate, enhancement of rectal absorption □ Coadjuvants—phytate, tripolyphosphate, adjuvant action in the presence of α-glycerophosphate □ Bioavailability—cefoxitin, dogs □ Inhibitors—disodium 4,4′-diisothiocyano-2,2′-disulfonate stilbene and calcium chloride

Rectal administration of drugs is becoming more popular due to the convenience of the suppository dosage form. Some anti-inflammatory drugs such as indomethacin (1–3) are currently administered as suppositories in clinical therapy. It has also been suggested (4, 5) that certain drugs which are metabolized by the liver (first-pass effect) after oral administration may have a higher systemic bioavailability when administered rectally.

In our attempt to find novel adjuvants which enhance rectal absorption of drugs poorly absorbed from the rectal compartment due to their high water solubility, we initially examined compounds with chelating activity. The potential link between chelation and adjuvant action was first recognized when the weak chelating agent acetoacetic acid was found to enhance the uptake of sulfadimethoxine into erythrocytes (6). We have since reported that other weak chelating agents such as salicylate analogues (7–9), enamine derivatives of β-diketones (10, 11), and glyceryl derivatives of acetoacetate (12) also effectively enhance drug absorption.

In the present study, we examine the absorption-promoting ability of phosphate derivatives since phosphate is generally known to have chelating ability. Sodium cefoxitin was used as a model water-soluble drug. The adjuvant action of disodium DL-α-glycerophosphate (I), sodium tripolyphosphate, and sodium phytate on the rectal absorption of cefoxitin in rats and dogs is reported. The adjuvant action of glycerol, sodium chloride, and disodium phosphate on rectal absorption of sodium cefoxitin in rats is also described. The ability of several of the compounds described above to act as coadjuvants, agents which promote drug absorption only when their own permeation through the membrane is enhanced by the presence of another absorption-promoting compound, was examined, and speculations concerning the mechanism of adjuvant and coadjuvant action were made.

EXPERIMENTAL SECTION

Materials—Disodium DL-α-glycerophosphate (I)[1], sodium tripolyphosphate[1], and sodium phytate[1] were analytical grade. Sodium cefoxitin[2] was used as obtained from the manufacturer.

Procedures—Sprague-Dawley male rats (weight, 200–225 g) were fasted, with water available, for 16 h prior to experimentation. During the experiments, rats were anesthetized with sodium pentobarbital (60 mg/kg) by intraperitoneal injection and kept on a 38°C surface. Drugs were administered rectally in microenema formulations with dosage volumes of ~ 100 μL/100 g of body weight. The initial pH of the microenema was adjusted to 5.8–6.3 with hydrochloric acid when necessary. To avoid leakage of the solution after administration, the rat anus was ligated with thread. Blood samples were taken from the jugular vein at designated time intervals with a syringe treated with 3% sodium citrate. Plasma samples were collected by centrifugation.

In experiments in which an *in situ* loop was used, the rat rectum was ligated with thread ~4 cm from the anus before administration of a microenema. The *in situ* loop was excised at 1.5 h after microenema administration (wet weight of tissue was 438.4 ± 41.3 mg measured after the experiments). The loop was rinsed with 8 mL of distilled water, and the total volume of the collected rinse solution was adjusted to 10 mL with distilled water. The amount of cefoxitin present in the solution was determined.

Ten male beagle dogs (weight, 9.5–12.0 kg) were also fasted, with water available, for 16 h prior to the experiments (crossover design). Drugs with particle sizes ranging from 72 to 144 μm were administered as 1-g suppositories, prepared with triglyceride suppository base[3] as described previously (12). Blood samples were taken from the jugular vein at designated time intervals.

An *in vitro* study of cefoxitin release from suppositories was carried out at 37°C. One-gram suppositories containing 0.45 mM cefoxitin with I, tripolyphosphate, and/or phytate (see Table II for formulations) were placed in 3 mL of a stirred saline solution. Samples were drawn from the beaker at designated intervals to determine the amount released.

Assays—Assays of cefoxitin were performed by the HPLC method described by Nishihata *et al.* (13).

RESULTS AND DISCUSSION

In a previous study (13), we found that sodium cefoxitin was poorly absorbed by the rat rectum. Plasma cefoxitin levels were undetectable (<0.22 μM) or very low (0.22–0.88 μM) after rectal administration of a microenema containing 44 mM cefoxitin prepared with distilled water. Coadministration of disodium DL-α-glycerophosphate (I) with cefoxitin increased the plasma cefoxitin levels significantly (Table I). A dose–response relationship was apparent since an increase in the concentration of I in the microenema caused an increase in plasma cefoxitin levels.

Glycerol was not as effective in promoting rectal cefoxitin absorption as did I (Table I). For example, 1.09 mmol of glycerol per kg of rat body weight had a similar effect on plasma cefoxitin levels as 103 μmol of I, a 10-fold difference. The sodium ion and phosphate moiety in I may, therefore, be actively involved in the adjuvant effect of the compound.

To investigate the adjuvant activity of the phosphate moiety, the adjuvant action of disodium phosphate, sodium tripolyphosphate, and sodium phytate was studied. Sodium tripolyphosphate and sodium phytate (dose, 0.026

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Merck & Co. Inc., Rahway, N.J.
[3] Witepsol H-15; Chemishe Werk, Witten, Federal Republic of Germany.

0022-3549/84/1100-1523$01.00/0
© 1984, American Pharmaceutical Association

Table I—Effect of α-Glycerophosphate (I), Glycerol (IV), Phosphate (V), Tripolyphosphate (VI), Phytate (VII), and Sodium Chloride (VIII) on Rectal Absorption of Cefoxitin [a]

Dosage Form	Dose of Additive, μmol/kg						Cefoxitin [b]		
	I	IV	V	VI	VII	VIII	Plasma Peak Levels, μM	[AUC], μM·min [c]	Bioavailability, % [d]
Intravenous injection [e]	0	0	0	0	0	0	—	2991 ± 342	100
Microenema	0	0	0	0	0	0	0.31 ± 0.30	61 ± 48	2.0
	0	1090	0	0	0	0	1.36 ± 0.53	122 ± 42	4.1
	103	0	0	0	0	0	2.29 ± 0.62 [f]	185 ± 54	6.2
	258	0	0	0	0	0	7.51 ± 1.80 [f]	756 ± 113 [f]	25.3
	515	0	0	0	0	0	13.43 ± 2.29 [f]	1274 ± 196 [f]	42.6
	0	0	138	0	0	0	0.58 ± 0.32	96 ± 57	3.2
	0	0	0	54	0	0	2.02 ± 0.49 [f]	153 ± 39	5.1
	0	0	0	0	26	0	1.79 ± 0.69 [f]	198 ± 26 [f]	6.6
	103	0	69	0	0	0	3.51 ± 117 [f]	472 ± 93 [f,g]	15.8
	103	0	0	27	0	0	15.93 ± 3.69 [f,g]	1345 ± 217 [f,g]	44.9
	103	0	0	0	13	0	17.60 ± 3.13 [f,g]	1618 ± 230 [f,g]	54.1
	0	0	0	0	0	520	2.38 ± 0.78 [f]	192 ± 65	6.4
	103	0	0	0	0	520	6.64 ± 1.60 [f,g]	546 ± 121 [f,g]	18.3
	258	0	0	0	0	520	15.20 ± 3.02 [f,g]	1548 ± 276 [f,g]	51.8

[a] A dose of 33.3 μmol/kg administered as microenemas (100 μL/100 mg) in rats. [b] Values are mean ± *SD*; $n \geq 4$. [c] $[AUC]_{0-240}$ min. [d] [AUC]rectal administration × (dose) intravenous injection × 100/[AUC]intravenous injection × (dose) rectal administration. [e] Intravenous injection of 33.3 μmol/kg administered in saline. [f] $p < 0.001$; Student's *t* test *versus* the results obtained after rectal administration without any additive. [g] $p < 0.001$ *versus* with I alone.

mmol/mL/kg, each contributing 0.27 mmol of sodium to the microenema) weakly enhanced the cefoxitin plasma levels, whereas the presence of disodium phosphate (0.138 mmol/mL/kg; contributing 0.27 mmol of sodium to the microenema) did not enhance cefoxitin absorption (Table I). In a sequential study in which I was coadministered with either sodium tripolyphosphate or sodium phytate in a cefoxitin microenema, the cefoxitin plasma levels were enhanced to a much greater degree than when I was administered alone (Table I). Although the coadministration of disodium phosphate with I only slightly enhanced the plasma cefoxitin level above that obtained with I alone (Table I), this enhancement is worth noticing since disodium phosphate itself had no effect on drug absorption. These results therefore suggest that once the transport of disodium phosphate and particularly sodium tripolyphosphate and sodium phytate through rectal epithelial cells is increased by the presence of I, the adjuvant ability of these phosphate derivatives can be realized.

In male beagle dogs, results were obtained which exhibited a similar trend as observed in the rat studies. The relative bioavailability of cefoxitin [measured by comparing the area under the curve from 0 to 240 min (AUC_{0-240}) of cefoxitin plasma levels after rectal administration to that after intravenous injection] in the absence of any adjuvant in the suppository was <5% (Table II). The presence of I in the suppositories administered to the dogs enhanced the plasma cefoxitin levels (Table II). Administration of either tripolyphosphate or phytate in a suppository containing cefoxitin only slightly enhanced the plasma cefoxitin levels. However, coadministration of I with either tripolyphosphate or phytate in a cefoxitin suppository produced substantially higher cefoxitin plasma levels than when either I or the coadjuvants was administered alone (Table II). Since >95% of the cefoxitin was released from each suppository within 20 min, it is unlikely that the release process was an important factor in this absorption study.

To determine what effect the sodium moiety had in the absorption-promoting ability of I, the adjuvant action of I and sodium chloride was compared in rats. The presence of 0.52 mmol of sodium chloride in a cefoxitin microenema did not have as great an effect on cefoxitin rectal absorption as did 0.258 mmol of I (also contributing 0.52 mmol of sodium/mL) (Table I). The strong adjuvant action of I is therefore not due solely to the sodium content. It is interesting to note that coadministration of sodium chloride with 0.258 mmol of I produced greater cefoxitin plasma levels than those obtained when only 0.258 mmol of I without sodium chloride was administered (Table I). Sodium chloride may, therefore, be effectively acting as a coadjuvant. Studies in which the effect of sodium ion on drug absorption are investigated are currently ongoing in our laboratory.

The exact physicochemical interactions that take place between I and the rectal membrane and that are involved in the enhancement of membrane permeability to cefoxitin and phosphate coadjuvants are not clearly understood. However, the inhibitory effect of disodium 4,4′-diisothiocyano-2,2′-disulfonate stilbene (II) on the enhancing action of I possibly suggests involvement of the membrane protein fraction. Compound II is known to inhibit anion transport through the erythrocyte membrane (14) by interacting with the amino group in the protein fraction (15). The disodium DL-α-glycerophosphate-enhanced disappearance of cefoxitin from the rectal *in situ* loop was suppressed when II was present in the microenema (Table III). Therefore, an interaction may occur between the adjuvant phosphate moiety and the membrane protein fraction, causing a conformational change in the protein. This change in protein structure ensuing from the interaction with I most probably does not result in membrane damage caused by chelation since the adjuvant effect of I on cefoxitin disappearance from a rectal *in situ* loop was only slightly suppressed by calcium chloride (Table III).

When calcium chloride was administered with either sodium tripolyphosphate or sodium phytate without I into a rectal *in situ* loop, the ability of both coadjuvants to enhance the disappearance of cefoxitin was significantly inhibited (Table III). The adjuvant action of these coadjuvants was not affected by coadministration of II (Table III). From these findings, we can speculate that the apparent adjuvant activity of sodium tripolyphosphate and sodium phytate when administered without I may depend primarily on chelating activity. In this study, the enhancing action of phytate was found to be similar to that of EDTA (16). EDTA, a strong chelating agent which has been shown to enhance drug absorption from the small intestine (17) and rectum (16) in rats, has also been found to cause solubilization of protein from erythrocyte ghosts (18). Consequently, the ability of tripolyphosphate and phytate to enhance rectal cefoxitin absorption when administered without I may be a result of membrane damage.

In a previous report by Nishihata *et al.* (12), the difference in the adjuvant action of glyceryl esters of acetoacetate was found to be due, partly, to the

Table II—Effect of α-Glycerophosphate (I), Tripolyphosphate, and Phytate on the Rectal Absorption of Cefoxitin [a]

Dosage Form	Dose of Additives, mmol/body			Cefoxitin, Mean ± *SD*		
	I	Tripolyphosphate	Phytate	Plasma Peak Levels, μM	[AUC], μM·min [b]	Bioavailability, % [c]
Intravenous injection [d]	0	0	0	—	2011 ± 426	100
Suppository	0	0	0	2.0 ± 0.91	291 ± 73	3.6
	1.03	0	0	10.4 ± 2.4 [e]	1519 ± 232 [e]	19.0
	1.03	0.054	0	21.8 ± 3.2 [e]	2458 ± 335 [e]	30.6
	1.03	0	0.026	24.3 ± 3.6 [e]	2883 ± 437 [e]	35.9
	0	0.135	0	3.2 ± 0.80	422 ± 146	5.3
	0	0	0.065	3.9 ± 0.96 [e]	519 ± 173 [f]	6.5

[a] At a dose of 0.45 mmol/dog administered as 1-g suppositories in dogs; crossover design with $n = 10$. [b] Values are $[AUC]_{0-240}$ min. [c] [AUC]rectal administration × (dose) intravenous injection × 100/[AUC]intravenous injection × (dose) rectal administration. [d] 50 mg of cefoxitin/dog was administered in saline. [e] $p < 0.001$; Student's *t* test, relative to results obtained after administration of suppositories without adjuvant. [f] $p < 0.05$; Student's *t*-test, relative to results obtained after administration of suppositories without adjuvant.

Table III—Effect of 4,4'-Diisothiocyano-2,2'-disulfonate Stilbene (II) and Calcium Chloride (III) on the Enhancement of Plasma Cefoxitin Levels and on Cefoxitin Disappearance from the Rat Rectal *In Situ* Loop by α-Glycerophosphate (I), Sodium Tripolyphosphate, and Sodium Phytate [a]

Adjuvant Concentration	Additive Concentration	Percent Disappearance of Cefoxitin at 1.5 h	$[AUC]_{0-30}$, μM·min
None	None	5.4 ± 5.8	<13[b]
I (205 mM)	None	38.7 ± 8.1	119 ± 28
	II (1 mg/mL)	16.8 ± 6.3[b]	52 ± 11[b]
	III (180 mM)	25.2 ± 5.3	99 ± 18
Tripolyphosphate (108 mM)	None	18.5 ± 4.2	61 ± 11
	II (1 mg/mL)	21.9 ± 7.1	66 ± 9
	III (180 mM)	7.8 ± 3.2[b]	<13[b]
Phytate (53 mM)	None	21.8 ± 5.1	67 ± 8
	II (1 mg/mL)	22.5 ± 5.4	77 ± 12
	III (180 mM)	7.6 + 3.9[b]	<13[b]

[a] Microenemas contained 15 mg of cefoxitin/mL and had a dosage volume of 100 μL/100 g of rat body weight; $n \geq 3$. [b] $p < 0.001$ *versus* adjuvant without additive.

difference in their ability to permeate the rat rectal tissue. In the present study, we found that the coadjuvants tripolyphosphate and phytate substantially enhanced the absorption of cefoxitin only after their own permeation of the membrane was apparently enhanced by I. Thus, we speculate that adjuvant permeation of the mucosal membrane may be mechanistically necessary before enhanced compound absorption across the rectal membrane can occur.

In conclusion, the enhancing action of I on rectal cefoxitin absorption appears to depend on the affinity of I to the protein fraction in the mucosal membrane. The weak enhancing ability of the phosphate coadjuvants when administered with cefoxitin alone may involve chelating activity and possible damage to the rectal mucosa. However, the large increase in drug absorption after coadministration of I with either chelating agent, tripolyphosphate or phytate, is not just a consequence of the added effect of the enhancing action of I and chelation. These phosphate derivatives apparently have a strong adjuvant efficacy which can be realized only when their own permeation of the mucosal membrane is enhanced by adjuvants such as I.

REFERENCES

(1) K. C. Kwan, G. O. Breault, E. R. Umbenhauer, F. G. McMahon, and D. E. Duggan, *J. Pharmacokinet. Biopharm.*, **4,** 255 (1976).
(2) V. Wright and M. Roberts, *Clin. Med.*, **80,** 12 (1973).
(3) E. C. Huskisson, R. T. Taylor, L. Bureston, P. Chater, and E. D. Hart, *Ann. Rheum. Dis.*, **29,** 393 (1970).
(4) A. G. de Boer, D. D. Breimer, H. Mattie, J. Pronk, and J. M. Gubbens-Stibbe, *Clin. Pharmacol. Ther.*, **20,** 701 (1979).
(5) A. G. de Boer, J. M. Gubbens-Stibbe, and D. D. Breimer, *J. Pharm. Pharmacol.*, **33,** 50 (1981).
(6) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.*, **27,** 1740 (1979).
(7) T. Nishihata, J. H. Rytting, T. Higuchi, and L. Caldwell, *J. Pharm. Pharmacol.*, **33,** 334 (1981).
(8) T. Nishihata, J. H. Rytting, and T. Higuchi, *J. Pharm. Sci.*, **71,** 865 (1982).
(9) T. Nishihata, J. H. Rytting, and T. Higuchi, *J. Pharm. Sci.*, **71,** 869 (1982).
(10) A. Kamada, T. Nishihata, S. Kim, M. Yamamoto, and N. Yata, *Chem. Pharm. Bull.*, **29,** 2012 (1981).
(11) S. Kim, A. Kamada, T. Higuchi, and T. Nishihata, *J. Pharm. Pharmacol.*, **35,** 100 (1983).
(12) T. Nishihata, S. Kim, S. Morishita, A. Kamada, N. Yata, and T. Higuchi, *J. Pharm. Sci.*, **72,** 280 (1983).
(13) T. Nishihata, H. Takahagi, M. Yamamoto, H. Tomida, J. H. Rytting, and T. Higuchi, *J. Pharm. Sci.*, **73,** 109 (1984).
(14) Z. I. Cabantchik, P. A. Knauf, and A. Rothstein, *Biochim. Biophys. Acta*, **515,** 239 (1978).
(15) J. A. F. op den Kamp, in "Membrane Structure," J. B. Finean and R. H. Michell, Eds., Elsevier/North-Holland, Biomedical Press, Amsterdam, 1981, p. 86.
(16) T. Nishihata, J. H. Rytting, and T. Higuchi, *J. Pharm. Sci.*, **70,** 71 (1981).
(17) H. Kunze, G. Rehbock, and W. Vogt, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **273,** 331 (1972).
(18) J. A. Reynolds and H. Trayer, *J. Biol. Chem.*, **246,** 7337 (1971).

ACKNOWLEDGMENTS

This work was supported in part by a grant from $INTER_x$, Merck Sharp & Dohme Research Laboratories. The authors also acknowledge support provided by the Center for Biomedical Research, The University of Kansas. The authors thank M. Dillsaver for useful comments and technical assistance.

Physiological Model for Distribution of Sulfathiazole in Swine

JOHN DUDDY [‡§], THOMAS L. HAYDEN [‡], DAVID W. A. BOURNE [*¶x], WILLIAM D. FISKE [*], IRMA H. BENEDEK [*], DOROTHY STANLEY [‡], ALBERTO GONZALEZ [‡], and WILLIAM HEIERMAN [‡]

Received August 29, 1983, from the [*] *College of Pharmacy and the* [‡] *Department of Mathematics, University of Kentucky, Lexington, KY 40506.* Accepted for publication December 19, 1983. Present addresses: [§]Department of Mathematics, De Paul University, Chicago, IL 60614 and [¶] Department of Pharmacy, University of Queensland, St. Lucia, 4067, Australia.

Abstract □ A physiological flow model was developed for the distribution of sulfathiazole residues in various tissues in swine. The approach was compartmental, in which the compartments and equilibrium constants had physiological meaning. Differential equations were developed, and appropriate parameter values and initial conditions were substituted and solved by a fourth-order Runga-Kutta technique. Simulation values corresponded with the experimentally determined concentration values in plasma and kidney, liver, muscle, fat, and heart tissues.

Keyphrases □ Sulfathiazole—physiological flow model, distribution, swine □ Physiological flow model—sulfathiazole, swine, distribution □ Distribution—physiological model, sulfathiazole, swine

Allergic reaction and increasing bacterial resistance are possible side effects resulting from human consumption of meat derived from animals given antibiotics (1). Consequently, regulations have been promulgated by the Food and Drug Administration setting tolerance levels for antibiotics and other drugs in slaughtered animals (2). Mathematical models which predict the residual amounts of a given drug in various organs at various times after administration are therefore important

0022-3549/84/1100-1525$01.00/0

© 1984, American Pharmaceutical Association