[Chem. Pharm. Bull. 25(11)3103—3106(1977)]

UDC 615.356 : 577.164.12.011.5.014.4.033

More Clarifications concerned with the Photodynamic Action of Riboflavine on the Intestinal Absorption of Drugs in the Rat

Fakhreldin S. Ghazy,[1a] Toshikiro Kimura, Shozo Muranishi,[1b] and Hitoshi Sezaki[1b,c]

Drug Research and Control Centre[1a] and Faculty of Pharmaceutical Sciences, Kyoto University[1b]

(Received March 17, 1977)

Investigation of the effect of riboflavine photolysates on the intestinal absorption of drugs has been pursued. Absorption studies were done using *in situ* recirculation technique. In the presence of riboflavine photolysates the intestinal absorption of phenol red was found to be pH dependant. The colon and rectal absorption of phenol red was promoted by the presence of riboflavine photolysates. No detectable effect of riboflavine photolysates on the intestinal absorption of moderately and rapidly absorbed drugs could be seen. There was no significant effect on the release of intestinal protein, contrary to the surface active agents where they produce undesirable side effects. Riboflavine photolysates modified the membrane permeability by decreasing its resistance to the passage of drug molecules known to be absorbed with great difficulty.

Keywords——riboflavine; photolysates; intestinal absorption; phenol red; moderately and rapidly absorbed drugs; protein release

In previous papers we have studied the influence of riboflavine (RF) photolysates on the intestinal absorption of certain drugs and their mechanism of action. It was found that, intestinal absorption of poorly absorbable compounds was greatly enhanced when the absorption experiments were done in the presence of RF and light.[2] A reversible effect of RF photolysates on membrane permeability was demonstrated. In contrast, intestinal absorption of D-glucose, an actively transported drug, was totally not affected. Moreover, investigation of the mechanism of action revealed an interaction between RF photolysates and phosphatidylcholine (PC), a major constituent of the phospholipids of the intestinal membrane, hence, change in membrane premeability due to disorganization of phospholipid molecules could be expected.[3]

In further experiments we attempted to clarify in more details the photodynamic action of RF on biological membranes.

Experimental

Reagents——All the chemicals were reagent grade unless otherwise specified. Bovine serum albumin was purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A.).

Animal Experiments——Male Wistar strain rats 150—200 g were used. The source of light, perfusion solutions and small intestinal absorption procedures have been previously mentioned[2] unless otherwise indicated.

Colon and Rectal Absorption——Animals were prepared as previously described.[2] The colon was cannulated near the ileocecal junction and at the end of the anus. Perfused solution volume was 20 ml. After recirculation, the following procedures were completed as previously mentioned.[2]

Preparation of Everted Rat Intestinal Sacs——They were prepared according to the method of Feldman *et al.*[4] Sacs were individually incubated in mucosal solution at 37° consisting of 20 ml of 0.1 mM RF in pH 6.5 phosphate buffer and it was oxygenated continuously with a mixture of 95% O_2—5% CO_2. Protected

1) Location: *a) Drug Research and Control Centre, P.O. Box 29, Cairo, Egypt; b) Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto; c)* To whom inquiries should be directed.
2) F.S. Ghazy, T. Kimura, S. Muranishi, and H. Sezaki, *J. Pharm. Pharmac.*, **27**, 268 (1975).
3) F.S. Ghazy, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 2335 (1976).
4) S. Feldman, M. Reinhard, and C. Willson, *J. Pharm. Sci.*, **62**, 1961 (1973).

experiments were shielded from light by aluminium foil, while the exposed were individually irradiated with 60 W white light placed at 15 cm above the incubation beaker. An electric fan was used to prevent elevation of temperature due to irradiation. A 1.0 ml samples were taken after one and two hours.

Analytical Methods——Phenol red was determined by appropriate dilution of the samples with 1 N NaOH and their extinction was measured at 550 nm.

Isonicotinic Acid and Isoniazide——Isoniazide was oxidized to isonicotinic acid by the addition of bromine water, then, sufficient $NaNO_2$ was added and isonicotinic acid was determined by the method of Nielsch and Giefer.[5]

Salicylic and Benzoic Acids——They were determined according to the method described by Schanker *et al.*[6]

Methylparaben——A 7.0 ml of chloroform were added to 3.0 ml sample of the intestinal perfusate. The mixture was shaken for 15 min and then centrifuged. A 5.0 ml aliquot of the chloroform phase was shaken with 5.0 ml of 0.1 N NaOH for 15 min then centrifuged and the extinction of the aqueous phase was measured at 294 nm.

Protein Determination——This was determined by the method of Lowry *et al.*[7] with bovine serum albumin as standard.

Results and Discussion

Intestinal Absorption of Phenol Red as a Function of pH and Presence or Absence of Riboflavine Photolysates

Luminal pH of the intestine is known to influence the intestinal absorption of drugs to a considerable extent. In the current study, it was of interest to investigate the influence of changing the pH in the presence or absence of RF photolysates on the intestinal absorption of phenol red as a model of the poorly absorbable compounds. Solutions of phenol red and RF were prepared in phosphate buffer at various pH values of 4.5, 6.5 and 8.0. Fig. 1 shows that, the intestinal absorption of phenol red in the presence of RF photolysates was greatly affected by changing the pH of the perfused solution. Increasing the pH from 4.5 to 8.0 was accompanied by an approximately 2 and 4-fold increase in the absorption of phenol red respectively. In contrast, there was almost no effect of changing the pH on phenol red absorption when the experiments were shielded from light. Since RF solution is more stable at the lower than higher pH values as it was found that the rate of photobleaching of RF at pH 5 is slow, but increases about 2.5-fold at lower pH to a maximum of approximately 3, then falls again. Above pH 5 the rate increases about 30-fold, then falls off again at pH values above 9,[8] consequently, the proposed alteration in membrane permeability is expected to be more pronounced at the higher pH values where the photolysates of RF are considered to be more.

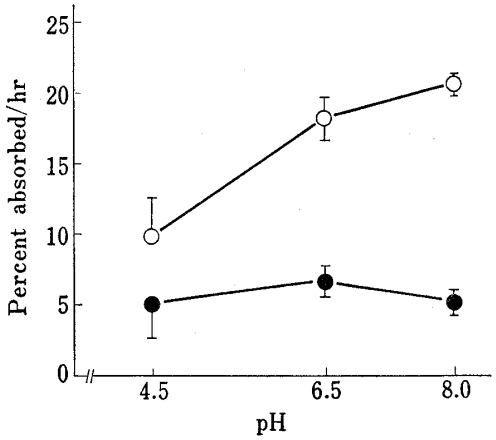

Fig. 1. Intestinal Absorption of Phenol red as a Function of pH and Presence or Absence of Riboflavine Photolysates

○, Exposed; ●, Protected.

Effect of Riboflavine Photolysates on the Absorption of Phenol Red from Other Site

Since it is known that, there is regional difference in the passage of drug molecules through the gastrointestinal barrier, we have intended to study the influence of RF photolysates on the colon and rectal absorption of phenol red. As is clear in Table 1 a significant

5) V. W. Nielsch and L. Giefer, *Arzneim.-Forsch.*, 9, 636 (1959).
6) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmac. Exptl. Therap.*, **120**, 528 (1957).
7) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
8) W.L. Cairns and D.E. Metzler, *J. Am. Chem. Soc.*, 93, 2772 (1971).

Table I. Effect of Riboflavine Photolysates on the Colon and Rectal Absorption of Phenol Red[a]

Condition	% absorbed in one hour[b]
Protected	2.9±1.1
Exposed	11.2±3.3

a) Phenol red (50 μg/ml) was dissolved in 0.1 mM solution of riboflavine in pH 6.5 phosphate buffer.
b) The percent absorbed in one hour expressed as the mean of at least three determinations±S.D.

enhancement in the absorption of phenol red in the presence of RF and light occurred. This result demonstrates change in permeability of the colon and rectum to the passage of phenol red molecules. Also, it illustrates that RF photolysates have influenced the absorption of a poorly absorbable compound not only from the small intestine, but also from a different absorption site.

Effect of Riboflavine Photolysates on the Intestinal Absorption of Moderately and Rapidly Absorbed Drugs

A significant effect of RF photolysates on the intestinal absorption of poorly absorbable compounds was well demonstrated.[2] Therefore, it was of importance to further investigate whether they have an influence on the intestinal absorption of moderately and rapidly absorbed drugs. Table 2 illustrates that the photolysates of RF have no influence on the intestinal absorption of isoniazide and isonicotinic acid as moderately absorbable drugs, and salicylic acid, benzoic acid and methylparaben as rapidly absorbable compounds. These results are of great interest, while the intestinal absorption of poorly absorbable compounds was promoted by the presence of RF photolysates, the absorption of moderately and rapidly absorbed drugs was completely not affected. This can be ascribed to that, the rate-limiting step in the absorption of the above-mentioned compounds is not the mucosal membrane since they are freely pass through the membrane, and their absorption is mostly influenced by blood flow and other factors at the serosal side. Furthermore, histological studies could not demonstrate any detectable change between treated (with RF photolysates) and untreated membrane, and the effect of RF photolysates was reversible,[3] consequently, no damage to the intestinal membrane can be expected. Therefore, disorganization of the phospholipids of the mucosal barrier[3] can not be expected to cause a significant change to drug molecules known to pass freely under normal physiological conditions across the intestinal mucosa. In contrast, the rate-limiting step in the absorption of the poorly absorbable compounds

Table II. Effect of Riboflavine Photolysates on the Intestinal Absorption of Moderately and Rapidly Absorbed Drugs[a]

Drug	Concn (mM)	% absorbed in one hour[b]	
		Protected	Exposed
Moderately absorbed:			
Isoniazide	0.1	57.63±5.03	51.74±6.62
Isonicotinic acid	0.1	33.68±6.77	36.21±9.58
Rapidly absorbed:			
Salicylic acid	1.0	71.87±5.45	81.88±4.16
Benzoic acid	50.0	77.74±8.00	79.58±6.98
Methylparaben	0.1	80.78±1.35	86.66±3.90

a) Each drug was dissolved in 0.1 mM solution of riboflavine in pH 6.5 phosphate buffer.
b) The per cent absorbed in one hour expressed as the mean of at least three determinations±S.D.

is considered to be the mucosal barrier, hence, disorganization of the membrane constituents should be expected to cause a remarkable change in their absorption. The present observation is in agreement with a recent publication of Meshali and Nightingale[9] where they have mentioned that the rapidly absorbed drugs would not expected to be sensitive to changes in membrane structure, and stated that it is difficult to see increase in the rate of transport of substances for which the gastrointestinal membrane ordinarily offers little resistance.

Effect of Riboflavine Photolysates on the Release of Intestinal Protein

The effect of riboflavine photolysates on phospholipids was shown in an earlier report.[3] Hence, we proceeded to investigate their influence on the release of membrane protein. As it can be seen in Table 3, neither the appearance of luminal protein nor the total amount of protein released from everted sacs show a significant difference whether the experiments were run in the presence or absence of light. Therefore, it can be considered that RF photolysates have no harmful effect on the integrity of the intestinal membrane in contrast to the effect of the surface active agents which was found to promote absorption of poorly absorbable drugs, but they produce some undesirable effects such as damage of the mucosal membrane,[10,11] release of the intestinal protein and inhibition of the intestinal absorption of actively transported drugs.[12]

Table III. Effect of Riboflavine Photolysates on the Release of Intestinal Protein

Time min	Condition	Protein released, mg[a]	
		Protected	Exposed
60	Luminal[b]	19.87±5.32	16.42±4.19[c]
60	Everted sacs	1.75±0.36	2.01±0.53[d]
120	Everted sacs	2.88±0.41	2.84±0.25

a) Calculated as bovine serum albumin.
b) A 40 ml of 0.1 mM riboflavine solution in pH 6.5 phosphate buffer was recirculated through the rat small intestine for one hour in the presence or absence of light, then washed out and the amount of protein released was determined.
c) Mean of at least 3 determinations±S.D.
d) Mean of 6 determinations±S.D.

Therefore, it can be concluded that the photolysates of riboflavine have demonstrated themselves as a new membrane modifier, where they modified the permeability of the intestinal membrane and made it less resistant to the passage of drug molecules known to be hardly absorbed. Furthermore, no damaging effect on the intestinal mucosa could be demonstrated.

Studies concerned with the effect of RF photolysates on the permeability of other natural membranes are in progress.

9) M.M. Meshali and C.H. Nightingale, *J. Pharm. Sci.*, **65**, 344 (1976).
10) T. Nadai, R. Kondo, A. Tatematsu, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1139 (1972).
11) T.S. Low-Beer, R.E. Schnieder, and W.O. Dobbins, *Gut*, **11**, 486 (1970).
12) J.A. Nissim, *Nature* (London), **187**, 308 (1960).