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The correlation between the *in vitro* and *in vivo* availability of salicylic acid for membrane transfer from polysorbate 20 solutions

J. H. COLLETT, R. WITHINGTON AND B. COX*

*Departments of Pharmacy and *Pharmacology, University of Manchester, Manchester M13 9PL, U.K.*

The presence of 1·0% w/v polysorbate 20 in solutions of salicylic acid reduces the absorption of the drug from the rat stomach. This effect is the resultant of two opposing effects of polysorbate 20 on salicylic acid absorption. Firstly, the surfactant reduces salicylic acid absorption by solubilization and secondly the surfactant tends to enhance salicylic acid absorption by an alternate unidentified effect. Knowing the magnitude of the absorption-enhancing effect the overall effect on salicylic acid absorption can be calculated using equations that describe the availability of salicylic acid for transfer across a Cellophane membrane from polysorbate solutions.

It has been demonstrated (Collett & Withington, 1973; Withington & Collett, 1973) that equations based on the pseudo-phase model of surfactant solutions can be used to describe the rate of dialysis of salicylic acid from solutions of polysorbates 20 and 80. Although the pseudo-phase model has been used qualitatively to explain the reduced *in vivo* absorption of drugs from surfactant solutions (e.g. Riegelman & Crowell, 1958; Whitworth & Yantis, 1967; Kakemi, Arita & Muranishi, 1965) quantitative correlations between solubilization and *in vivo* absorption data are not frequently reported (Yamada & Yamamoto, 1965; Yamada, Ichihashi & others, 1966). In this paper the effect of polysorbate 20 on the *in vivo* absorption of salicylic acid from the rat stomach is investigated. The aim of the work is to determine whether the equations describing the dialysis of salicylic acid from polysorbate solutions are applicable to *in vivo* absorption.

The rat was chosen as the experimental animal. There are basically two gastrointestinal regions at which drug absorption can be studied: the stomach and the small intestine. A fundamental difference between these sites is their physiological pH which is about pH 1–2 in the stomach and about pH 6 in the small intestine. If the absorption experiments are carried out on the stomach then salicylic acid solutions maintained at pH 1·0 by the presence of 0·1M hydrochloric acid can be used, and the use of a pH stat (Withington & Collett, 1973a) is avoided. Experimentally, the stomach can be prepared for absorption experiments more easily than the small intestine. For these reasons it was decided to measure absorption* from solutions of salicylic acid at pH 1·0 perfused through the stomach.

* The decrease in the amount of salicylic acid in the perfusion solutions during the course of an experiment is used as a measure of absorption.

MATERIALS AND METHODS

Materials

The reagents were described by Collett & Withington (1972). Albino, Wistar strain rats of either sex, 200–380 g, were given sugar cubes in place of the usual diet for 48 h before each experiment. This treatment avoided starving the rats while providing a stomach which was reasonably free from food particles at the start of an experiment.

Preparation of perfusion solutions

Other workers have reported the necessity of using isotonic solutions in drug absorption experiments (Pelzmann & Havemeyer, 1972; Kojima, Smith & others, 1972). The tonicity of all the solutions used in the present work was determined using a vapour pressure osmometer (Hewlett Packard 302 B), and adjusted appropriately with sodium chloride. Each solution had an osmotic pressure which was close to the osmotic pressure of the solvent, 0·1M hydrochloric acid. Sodium chloride, at a concentration of 2·5 mg ml^{-1}, was added to render them isotonic with normal saline.

Salicylic acid assay

Substances appearing in the perfusion solutions, probably derived from blood, interfered with the usual spectrophotometric assay for salicylic acid. A fluoroscopic technique was developed which was insensitive to the presence of blood and polysorbate 20. 1·0 ml samples of perfusion solution were diluted to 10·0 ml with pH 7·0 phosphate buffer solution and filtered through a Millipore filter (0·45 μm pore size) held in a Swinnex filter adaptor. Fluorescence of the samples was measured (Farrand spectrophotofluorimeter) at an excitation wavelength of 315 nm and an emission wavelength of 420 nm. Perfusion solutions of 0·1M hydrochloric acid were similarly assayed and the fluorescence intensity of these solutions was subtracted from the intensity determined with salicylic acid solutions. Concentrations of salicylic acid were determined from calibration curves which were prepared each time a series of solutions was assayed.

Operative procedure on rats

General anaesthesia was induced with urethane (25% w/v) given by intraperitoneal injection (1·0 ml per 100 g) and body temperature (measured rectally) was maintained at 34°. The stomach was exposed by a midline abdominal incision and polythene cannulae were inserted through the cardiac and pyloric sphincters via incisions in the oesophagus and duodenum respectively, and were ligated avoiding blood vessels serving the stomach. The stomach was washed by pumping (Watson Marlow H.R. Flow Inducer) normal saline into the oesophageal cannula and out through the duodenal cannula. Washing was continued during the remainder of the operation. A tracheotomy was also performed.

Heparin (500 units) was introduced into the external jugular vein via a fine polythene cannula and washed into the vein with 0·2 ml of saline from a 5·0 ml burette. Mean arterial blood pressure was measured with a Statham pressure transducer, via a cannula inserted into the external carotid artery, and recorded on a pen recorder (Devices Instruments Ltd.).

Procedure

After washing with normal saline the stomach was rinsed with 50 ml of the first perfusion solution (0·1M HCl). A further 20 ml of this solution was then recirculated through the stomach and 1·0 ml samples were removed 15 and 30 min after the commencement of recirculation. Samples were assayed for salicylic acid by the method described. After removal of the second sample, the stomach was rinsed with 50 ml of the next perfusion solution in the series (salicylic acid without or with 1% polysorbate 20) and the recirculation procedure repeated. Altogether three salicylic acid solutions (0·02 mg ml^{-1}), either with or without 1·0% w/v polysorbate 20, were perfused between three further perfusions of 0·1M HCl.

Experiments were terminated if the animal's blood pressure fell below 30 mm Hg.

RESULTS AND DISCUSSION

After 15 min perfusion the mean percentage absorption of salicylic acid from aqueous solutions was 18·4% ± 0·83 s.e. (n = 15), and from aqueous solutions containing 1·0% w/v polysorbate 20, 14·6%. ±1·23 s.e. (n = 13). Thus the presence of 1·0% w/v polysorbate 20 reduced salicylic acid absorption to about 79% of that observed in the absence of surfactant. After 30 min perfusion salicylic acid absorption was reduced to about 72% (from 26·6 ± 0·90 s.e. to 19·1 ± 1·23 s.e.). The significance of these differences, determined by Student's *t*-test, was after 15 min $P < 0{\cdot}02 > 0{\cdot}01$ and after 30 min $P < 0{\cdot}001$.

The concentration of salicylic acid in the aqueous phase of a 1·0% w/v polysorbate 20 solution at pH 1·0 can be calculated if the total salicylic acid concentration is known (Collett & Withington, 1973),

$$C_a = C_t \frac{V_t}{\frac{P^0 V_m}{V_a} + \frac{P^- V_m}{(1/f_i)-1 V_a} + 1 + \frac{1}{(1/f_i)-1} V_a} + 1 + \frac{1}{(1/f_i)-1} \quad .. \quad (1)$$

where C represents concentration, V volume and P partition coefficient; subscripts a, m denote aqueous and micellar phases and t is the sum of aqueous and micellar phases. Superscripts 0 and − denote unionized and ionized salicylic acid respectively. f_i is the fraction of salicylic acid ionized at any pH. When the total concentration was 0·02 mg ml^{-1}, as in these absorption experiments, the aqueous salicylic acid concentration was calculated to be 0·011 mg ml^{-1}. Thus if only aqueous salicylic acid was available for absorption and provided that the surfactant did not influence absorption by mechanisms other than solubilization, an average of 18·4% and 26·6% of 0·011 mg ml^{-1} should be absorbed from polysorbate 20 solutions after 15 and 30 min respectively. These figures are equivalent to 10·1% and 14·6% of the total salicylic acid concentration and are considerably lower than the corresponding experimentally determined values of 14·6% and 19·1% respectively.

The concept of drug availability from only the aqueous phase of surfactant solutions, that was successfully applied *in vitro*, appears to underestimate the extent of absorption occurring from surfactant solutions *in vivo*. A possible explanation for this is that in addition to reducing the amount of salicylic acid available for absorption, the surfactant enhances absorption by a secondary effect (Alexander & Trim, 1946; Levy, Miller & Reuning, 1966; Levy & Anello, 1968). Overall, the surfactant would

reduce the amount of salicylic acid absorbed, but to a lesser extent than calculations based solely on solubilization would suggest. In order to investigate the possibility that polysorbate 20 enhanced salicylic acid absorption by a mechanism other than solubilization, salicylic acid absorption from rat stomachs pretreated with polysorbate 20 was studied. Perfusion with normal saline was followed by 0·1M HCl, salicylic acid, 1% w/v polysorbate solution alone and then salicylic acid followed by HCl both twice.

A series of three salicylic acid solutions was perfused through the stomach in the usual way except that between the first and second solution a 1·0% w/v polysorbate 20 solution at pH 1·0 was substituted for 0·1M hydrochloric acid. Table 1 shows that the percentage of salicylic acid absorbed from the solution immediately after pretreatment with surfactant was consistently greater than from either of the other two salicylic acid solutions. The mean percentage increase in absorption, due to pretreatment with polysorbate 20, was 29·3% (n = 16) (calculated from the percentage increases

Table 1. *The effect of pretreatment with 1·0% w/v polysorbate 20 on the absorption of salicylic acid from the rat stomach.*

Animal number	Perfusion solution	Salicylic acid absorbed (%) 15 min	30 min	Increase in absorption due to pretreatment (%)[a] 15 min	30 min
1	Salicylic acid	11·3	18·4		
	Polysorbate 20	—	—		
	Salicylic acid	16·4	24·6	33	41
	Salicylic acid	13·3	17·4		
2	Salicylic acid	16·2	22·2		
	Polysorbate 20	—	—		
	Salicylic acid	21·6	26·5	25	18
	Salicylic acid	18·4	22·7		

[a] Increase in absorption is relative to the mean of the absorption from the first and last salicylic acid solutions perfused in each rat.

after 15 and 30 min in both of the rats used). If the increase in salicylic acid absorption causd by pretreatment with polysorbate 20 also occurs in the presence of the surfactant, then the overall effect on salicylic acid absorption will be the sum of this effect and the effect of solubilization. On the basis of solubilization alone, salicylic acid absorption from 1·0% w/v polysorbate 20 solution has been estimated to be 10·1 and 14·6% after 15 and 30 min respectively. If these values are adjusted to take into account the tendency of polysorbate 20 to increase salicylic acid absorption by 29·3%, then figures of 13·1% and 18·9% respectively are obtained which agree well with the experimentally determined values of 14·6 and 19·1%.

Polysorbate 20 appears to influence salicylic acid absorption by two distinct effects. The first of these is solubilization which, under the conditions of the experiment, was expected to reduce salicylic acid absorption from 26·6 to 14·6% of the total amount over a 30 min period. The second effect is an enhancement of absorption which, over a 30 min period, increased salicylic acid absorption by an average of about 30%. Summing the magnitude of these two opposing effects provided a reasonably good estimate of the total effect of polysorbate 20 on salicylic acid absorption.

It can be concluded that salicylic acid solubilized within surfactant micelles is not available for absorption *in vivo*. Consequently, equations derived to calculate the rate of dialysis of salicylic acid from surfactant solutions can also be used to calculate *in vivo* absorption rates of salicylic acid in the presence of polysorbate 20.

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