International Journal of Pharmaceutics, 64 (1990) 161–169
Elsevier

IJP 02149

Intestinal absorption of drugs. IV. The influence of taurocholate and L-cysteine on the barrier function of mucus

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(Received 17 February 1990)
(Accepted 25 March 1990)

Key words: Taurocholate; Cysteine, L-; Rheological properties; Barrier function; Mucus; Intestinal absorption; Lipophilic drug

Summary

The results in this report show that taurocholate (TC) is able to alter the rheological properties ('viscosity') of native porcine mucus in vitro. Below the critical micelle concentration (CMC) the viscosity reduction is dependent on the concentration of TC; above the CMC the viscosity of the mucus is not decreased further: this indicates that the effect of TC on the viscosity of the mucus is not the result of micellar solubilization. The effect of TC on the intraluminal release of mucus components (hexoses) in the small intestine of the rat, is also concentration-dependent: below the CMC (about 8 mM) the hexose output is not altered, but above the CMC the hexose output is increased to a concentration-independent level. The mucolytic agent L-cysteine also reduces the viscosity of mucus in vitro and induces an increase in the hexose output in vivo. Absorption experiments with 10 mM TC in an isolated segment of the small intestine of the rat reveal that the disappearance rate of dantrolene (DA) and ketoconazole (KE) is reduced by TC, but apparently this reduction is almost proportional to the decrease in the fraction of the drug free in solution due to micellar solubilization. DA and KE are not solubilized by L-cysteine and absorption experiments with L-cysteine reveal that the absorption rate of these drugs is not affected by L-cysteine. These results indicate that TC and L-cysteine do not have a measurable or pronounced effect on the absorption barrier in vivo. An explanation for these results may be that, if the rate-limiting barrier to the transport of lipophilic drugs from the lumen to the serosa is located in the mucous layer, then the effects on the mucus by TC and L-cysteine must be neutralized by a compensatory mechanism, which is restoring the barrier function instantaneously, e.g. by the secretion of mucus from the goblet cells.

Introduction

The influence of bile salts on the transfer of solutes across the pre-epithelial diffusion barrier and the cellular membranes of the intestinal wall has been ascribed to a decreased thermodynamic activity caused by incorporation in micelles (Kakemi et al., 1970; Kimura et al., 1972; Feldman et al., 1973; Yamaguchi et al., 1986; Poelma et al., 1989, 1990) and to an alteration of the barrier function (Kakemi et al., 1970; Feldman et al., 1973). Experimental data reported in the literature indicate that the rate-limiting step in the absorption process of lipophilic solutes is the transfer across the aqueous diffusion barrier adjacent to the intestinal wall (Komiya et al., 1980). A part of this aqueous diffusion resistance is the mucous layer, but it is difficult to determine the

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exact contribution of mucus to this barrier function. Several effects of bile salts on the mucous layer have been described. Studies by Martin et al. (1976, 1978) indicate that bile salts are able to decrease the gel structure ('viscoelasticity') of mucus; besides, bile salts are capable of reducing the amount of mucus in the goblet cells (Yonezawa, 1977; Lewin et al., 1979); and finally, a stimulation of mucus production has been suggested (Lewin et al., 1979; Slomiany et al., 1987; Poelma et al., 1989). The consequences of these effects for the barrier function of the mucous layer in the absorption of lipophilic drugs, however, are not clear.

The present study was performed to examine the effect of taurocholate (TC) and the mucolytic agent L-cysteine (Sheffner, 1963) on the rheological properties and the barrier function of mucus. The influence of TC and L-cysteine on mucus was also evaluated by determining the intraluminal release of mucus components (hexoses) in a chronically isolated segment of the small intestine of the rat in vivo. The critical micelle concentration (CMC) of TC was determined in vitro and the relation between the CMC and the influence of TC on the mucous layer was evaluated. The influence of TC and L-cysteine on the absorption of two lipophilic drugs was studied – separately – in a chronically isolated intestinal segment of the rat. These experiments allowed us to gain insight into the role of mucus in the absorption kinetics of lipophilic drugs.

Absorption kinetics were evaluated on the basis of disappearance rates of the drug from the perfusion solution. The absorption rate of the drugs from solutions containing TC or L-cysteine was compared with the absorption of the drug from saline solutions in the same intestinal segment. Dantrolene (DA) and ketoconazole (KE) were chosen as model drugs.

Materials and Methods

Materials

DA and KE were gifts from Norwich Eaton Pharmaceuticals Inc., NY, U.S.A., and Janssen Pharmaceutica, Beerse, Belgium, respectively. TC was obtained from Sigma, St. Louis, U.S.A. L-Cysteine and glucose monohydrate were supplied by Merck, Darmstadt, F.R.G. All other chemicals were of analytical grade.

Mucus was collected from the small intestine of freshly slaughtered domestic pigs (from Encebe, Boxtel, The Netherlands) which had been starved for approx. 24 h. The mucus was gently scraped off the mucosal surface, divided into 5-ml aliquots and stored at −20 °C until the viscosity determinations were performed.

A pH-meter (type E632, Metrohm Herisau, Switzerland), a constant-temperature water bath (Thermomix 1420, B. Braun, F.R.G.), a spectrophotometer (Pye Unicam PU 8600, Cambridge, U.K.), HPLC system (model 710B Waters Intelligent Sample Processor and model 440 UV Absorbance Detector, Waters Associates, Milford, MA, U.S.A.), a peristaltic pump (VRX-22, Verder, Düsseldorf, F.R.G.), and a Ferranti-Shirley coneplate viscometer (Epprecht, Rheomat 15, Zürich, Switzerland) were used.

Critical micelle concentration (CMC) of TC

The CMC of TC was determined by two methods. Firstly, the CMC was assessed from the surface tension of phosphate-buffered isotonic saline solutions (PBS: 66 mM sodium phosphate, 88 mM sodium chloride; pH 7.4) with increasing concentrations of TC (1–40 mM). The surface tension of the various TC solutions was measured by a DuNouy tensiometer at 24 °C. Secondly, the

TABLE 1

HPLC methods for the determination of dantrolene (DA) and ketoconazole (KE)

Drug	Stationary phase	Eluent	Wavelength of detection (nm)
DA	LiChrosorb® RP8	acetonitrile/ phosphate buffer, pH 6.8, 45 : 55 (v/v)	405
KE	Chrom-Sep Hypersil/ODS®	acetonitrile/ water/ diethylamine 48 : 55 : 0.02 (by vol.)	254

solubility of DA in PBS (pH 7.4; approximately the pK_a of DA [Product Information, Norwich Eaton Pharmaceuticals], 37°C) with increasing concentrations of TC (2–40 mM) was determined. The CMC of TC was assessed from the solubility data by interpolation: the concentration of TC where the solubility of DA starts to deviate from the solubility in the buffer solution is defined as the CMC of TC. The solubility of DA in PBS, pH 7.4, and in the same medium with various concentrations of TC, was determined by shaking suspensions of DA for 24 h at 37°C. After centrifugation (30 min at $1000 \times g$), the concentration of DA in the supernatant was determined directly by HPLC (Table 1).

Determination of the thermodynamic activity after solubilization

The thermodynamic activity, and thus the driving force for absorption, of the model compounds during absorption experiments with 10 mM TC or 3% L-cysteine was assessed from the solubilization of the model compounds by 10 mM TC and 3% L-cysteine, respectively.

The solubility of DA was determined in PBS (pH 7.4, 37°C) as described above. The solubility of KE was determined in PBS at pH 6.5 (pK_a of KE [Product Information, Janssen Pharmaceutica]). The buffer solutions used for the solubility determinations were identical to the perfusion buffers in the absorption experiments.

The solubilization of DA and KE by 10 mM TC and 3% L-cysteine was determined by measuring the solubility of the respective compounds in the same buffer solutions as mentioned above with 10 mM TC or 3% L-cysteine, and the fraction of the drug free in solution was calculated.

Theoretical

The drug solution with TC micelles is considered to consist of two separate phases: (1) an aqueous phase with a fraction of the drug free in solution, and (2) a micellar phase with the remaining fraction of the drug solubilized in micelles.

The fraction of the drug solubilized in micelles (s) and that free in solution (f) is calculated from the solubility data with Eqns 1 and 2:

$$s = (C^+ - C^-)/C^+ \quad (1)$$

$$f = 1 - s \quad (2)$$

where C^+ is the solubility of the drug in the solution with micelles and C^- is the solubility in the same medium without micelles. It is assumed that s is constant and independent of the drug concentration for a particular concentration of micelles, and that the distribution of the solute between the aqueous phase and the micellar phase is not rate limiting; it is also assumed that only one type of micelle exists in the micellar solutions.

If the fraction of the drug free in solution is considered as the only driving force for absorption, it is expected that during absorption experiments with drug solutions the reduced concentration of the free drug due to micellar encapsulation will result in a proportional decrease of the absorption rate of the drug, provided that the absorption barrier is not affected by the micelles.

Influence of TC and L-cysteine on the mucous structure in vitro

In order to investigate the influence of TC and L-cysteine on the mucous barrier, the effect of TC and L-cysteine on the 'viscosity' of native mucus of porcine small intestine was studied in vitro.

Mucus possesses both viscous and elastic properties. The characterization of the viscosity of mucus has no real physical meaning, but is indicative for the forces which are responsible for the gel structure of the mucus. The viscosity of mucus was assessed by determining the relation between shear stress and shear rate by means of a cone-plate viscometer.

The mucus sample were defrosted over a period of 15 min at 37°C and used without further homogenisation or purification, since these procedures might reduce the gel properties of the mucus as was shown by Smart et al. (1984). A weighed sample (2.60 g) of the mucus was gently mixed with 150 µl of a saline (control) or a concentrated solution (in saline, pH 7.4) of the respective micelles so that in the mucus sample the required final concentration resulted. After mixing the

mucus sample was transferred between cone and plate of the viscometer. The mucus sample was allowed to equilibrate in a saturated atmosphere at 37 °C for 30 min. After adjustment of the shear rate, the shear stress was read after exactly 1 min. The procedure was performed both at increasing and decreasing shear rates. The viscous component of the mucus was assessed by determining the slope of the arbitrarily chosen linear region (a–b) at decreasing shear rates (Kearney and Marriott, 1986; see the rheogram in Fig. 2). The effect of various concentrations of TC (2–30 mM) on the viscosity of the mucus was compared to the control (saline) based on the same mucus batch and performed on the same day.

The results of the viscosity determinations were given as the relative viscosity (V). The relative viscosity is expressed as:

$$V = (\text{viscosity}_{\text{mucus + test solution}} / \text{viscosity}_{\text{mucus + saline}}) \times 100\% \quad (3)$$

Chronically isolated internal loop of the small intestine of the rat

In this study a chronically isolated intestinal loop in the rat, as described earlier (Poelma and Tukker, 1987), is used to study the effect of TC and L-cysteine on both the intraluminal release of hexoses and the absorption kinetics of DA and KE. The animal model offers the opportunity to perfuse an isolated segment of the intestine of the rat with a constant volumetric flow of drug solution under well-defined conditions in a conscious animal, and more than one experiment can be performed in the same animal over an extended period of time. Briefly, an intestinal segment of approx. 8 cm (approx. 15 cm proximal to the ileo-caecal junction) was isolated with intact blood supply. The loop remained in the peritoneal cavity. The perfusion solution could enter and leave the segment via two Delrin® cannulas through the abdominal wall. The head-tail connection of the remaining intestine was restored by end-to-end anastomosis. After surgery the rat was placed into a restriction cage and supplied with water and food. After recovery from the operation (2–4 days) the rat was ready for use in perfusion experiments.

Effect of TC and L-cysteine on the intraluminal release of hexoses in the chronically isolated internal loop

Before starting the perfusion, the intestinal loop was cleaned by rinsing for 30 min with isotonic saline at a flow rate of 1.0 ml/min. During perfusion experiments, the perfusion solution was pumped through a heat exchange device to bring the solution to body temperature just before entering the rat.

The influence of various concentrations of TC (ranging from 0 to 20 mM) on the mucous layer was determined by measuring the intraluminal release of hexoses (glucose equivalents) in the perfusion solution after a recirculating perfusion (volume, 60 ml; flow rate, 1 ml/min) during a 3 h period with the respective concentrations of TC in PBS, pH 7.4. The perfusions at each TC concentration were performed in various rats ($n = 4$–10) and the release of hexoses was standardized per unit intestinal length (cm). The influence of 3% L-cysteine on the intraluminal release of hexoses was determined in the same manner.

Hexoses in the perfusion solution were determined according to a slightly modified version of the method of François et al. (1962), as was described earlier (Poelma et al., 1989). Glucose monohydrate was used as a standard. Sodium taurocholate interfered with the assay; a standard addition method was used to circumvent this problem. L-Cysteine did not interfere with the assay.

Absorption experiments

The absorption kinetics of DA and KE were evaluated on the basis of disappearance rates of the drugs from the perfusion solution in the intestinal loop. The absorption of the drugs in the presence of 10 mM TC or 3% L-cysteine was compared in the same intestinal segment with the absorption of the drug from the same medium without TC or L-cysteine. Perfusions were performed in a recirculating mode (perfusion volume, 60 ml) at a rate of 1.0 ml/min.

The perfusions with DA were performed in PBS, at a pH of 7.4; for KE the pH of the perfusion solution was 6.5. The pH of the perfusion solution remained within narrow limits (± 0.1)

during the perfusion experiments. The concentration of the respective drugs in the perfusion solutions is below the saturation concentration of the respective drugs in the buffer solution. The perfusion solutions were freshly prepared shortly before starting the experiments. During the perfusion samples were taken from the perfusion solution at 0, 0.5, 1, 1.5, 2, 2.5 and 3 h after the start of the perfusion and directly analysed by HPLC (Table 1).

The disappearance of the respective model compound from the perfusion solution can be described by first-order kinetics. The time dependence of the concentration in the perfusion solution, C, can be expressed as:

$$\ln(C_t/C_0) = -k_{dis} \cdot t \quad (4)$$

where C_0 and C_t are the concentrations of the model compounds in the perfusion solution at time 0 and t, respectively. The disappearance rate constant k_{dis} (h^{-1}) was calculated from plots of $\ln(C_t/C_0)$ vs t by linear regression.

Evaluation of the absorption data

The disappearance rate constants are determined in a cross-over experimental scheme; therefore the effect of the additive on the absorption of the model drug can be described by the ratio (r_{obs}):

$$r_{obs} = k_{dis\ with\ additive}/k_{dis\ without\ additive} \quad (5)$$

Statistical evaluation

For the mean ratio r_{obs} the 95% confidence interval (CI) was calculated (two-sided t-test for one sample at the 5% level; Bolton, 1982).

The output of hexoses during perfusions with an additive was compared with that without an additive and the difference was statistically tested for significance (two-sided t-test for unpaired samples at the 5% level; Bolton, 1982).

Results and Discussion

Critical micelle concentration (CMC) of taurocholate

In order to assess the CMC of TC, the effect of the concentration of TC on the surface tension of the buffer solution was studied (Fig. 1). In the same figure, the effect of various concentrations of TC on the solubility of the lipophilic compound DA is demonstrated.

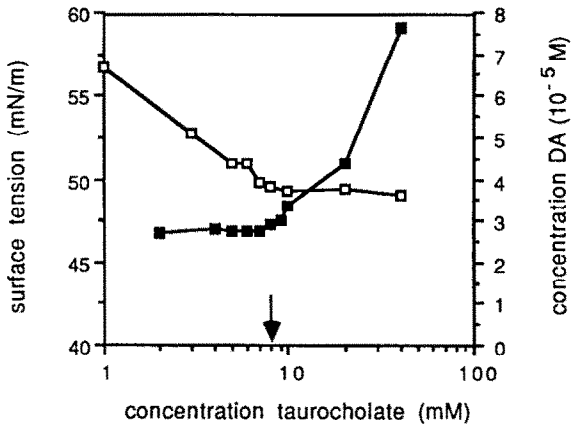

Fig. 1. Surface tension of TC solutions (phosphate-buffered saline, pH 7.4, 24°C) and solubility of DA in TC solutions (phosphate-buffered saline, pH 7.4, 37°C). (Open symbols) Surface tension of the TC solutions; (closed symbols) solubility of DA (average of two determinations); the arrow indicates the CMC of TC.

Both the measured surface tension and the solubilization of DA indicate a CMC of approx. 8 mM. At this concentration no further decrease of the surface tension takes place, and the solubilization increases from this concentration on linearly with the concentration of TC (Fig. 1). This inflection point corresponds with the CMC of TC (Attwood and Florence, 1983). The fact that the value for the CMC obtained in this study deviates from the data reported in literature (3–6 mM; Carey and Small, 1971) can be ascribed to different experimental conditions, such as pH, and the presence and concentration of inorganic anions (Ockner and Isselbacher, 1974).

Determination of the thermodynamic activity after solubilization

The solubilization of the model drugs by TC was determined by measuring the solubility of the drugs in solutions with 10 mM TC (concentration above the CMC). The fraction of the drug solubilized (s) and that free in solution (f) were calcu-

TABLE 2

Solubilization of dantrolene (DA) and ketoconazole (KE) by 10 mM taurocholate and 3% L-cysteine

Drug	Solution	Solubility (10^{-5} M) ±SD	n	s	f
DA	buffer	2.3±0.3	41	–	–
	10 mM TC	3.0±0.4	21	0.23	0.77
	3% L-cysteine	2.2±0.2	3	≈0.0	≈1.0
KE	buffer	1.3±0.2	13	–	–
	10 mM TC	2.7±0.4	10	0.52	0.48
	3% L-cysteine	1.3±0.1	3	0.0	1.0

s indicates the fraction of the drug solubilized; f denotes the fraction of the drug free in solution; n is the number of experiments.

lated from the solubility data using Eqns 1 and 2, and the results are listed in Table 2.

DA is solubilized by 10 mM TC to a lesser extent than KE, resulting in an s value of 0.23 vs 0.52 for KE. DA and KE are not solubilized by 3% L-cysteine: the solubility and the fraction of the drug free in solution (f) for both drugs with L-cysteine in the buffer solution is identical to the situation without L-cysteine in the medium ($f \approx 1.0$ and $s \approx 0.0$; Table 2).

Influence of TC and L-cysteine on the structure of mucus

The effect of TC and L-cysteine on the mucous structure was determined by assessing the relation between the shear stress exercised on the treated mucus sample and the corresponding shear rate.

An example of the relation between the shear stress and shear rate of the mucus sample after addition of a saline solution and after addition of TC up to a final concentration of 10 mM is given in Fig. 2. The viscosity of the mucus was assessed from the slope of the arbitrarily chosen linear region (a–b) of the rheogram. The results show that the molecular cohesion of the mucus is reduced after addition of 10 mM TC to the mucus sample.

The influence of increasing concentrations of TC on the mucous structure is illustrated in Fig. 3. The viscosity of the mucus treated with TC is reduced compared to the value of the control mucus sample (100%). As the concentration of TC reaches a value of approx. 8 mM, the viscosity is 65–70% of the control value and remains at this level even if TC is added to the mucus sample up to a final concentration of 30 mM. The viscosity determinations indicate a concentration-dependent effect of TC on the mucous layer below the CMC (approx. 8 mM; Fig. 1). At concentrations of TC above the CMC this concentration-dependent effect is not observed and TC does not decrease the viscosity of the mucus further; apparently, the effect of TC on the gel structure of the mucus is not the result of micellar solubilization of (lipophilic) mucus components.

The addition of 3% L-cysteine to the mucus sample also results in a reduction of the viscosity compared to the control mucus sample. A relative viscosity of 32 ± 4% (±SD, $n = 3$) compared to the control value is found.

The reduced consistency of the mucus due to the action of TC and L-cysteine does not necessarily mean that the barrier function to permeating drug molecules is also decreased. The 'microclimate' of mucus consists mainly of 'free' water which results in a low 'effective' viscosity (Kearney and Marriott, 1986) and diffusion coefficients comparable to those in pure water. This 'microviscosity' of the mucus will not be affected by TC and L-cysteine. The reduced cohesion of the glycoprotein molecules, however, may lead to a facilitated erosion of the mucous layer in vivo in the

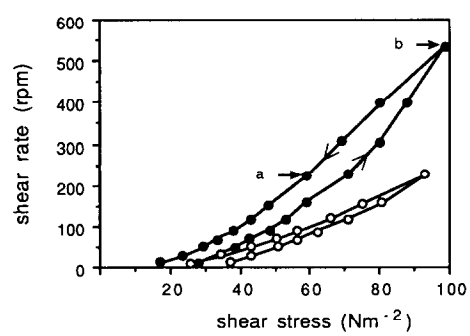

Fig. 2. Rheograms of mucus treated with saline and treated with TC. The viscosity of the mucus was defined in the study as the slope of the linear region (a–b) at decreasing shear rates. (Open symbols) Mucus treated with saline; (closed symbols) mucus treated with 10 mM TC.

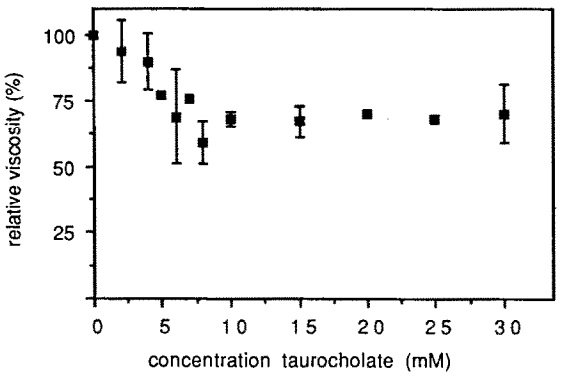

Fig. 3. Effect of TC on the relative viscosity of mucus. The vertical bars indicate the standard deviation ($n = 3$–13).

lumen, resulting in a reduced thickness of the mucous layer and subsequently an improved transfer of drug molecules across this diffusion resistance.

The influence of various concentrations of TC (ranging from 2 to 20 mM) on the mucous layer in the small intestine of the rat in vivo was determined by measuring the intraluminal release of hexoses (expressed as glucose equivalents) into the perfusion solution after a recirculating perfusion with the respective concentrations of TC. The

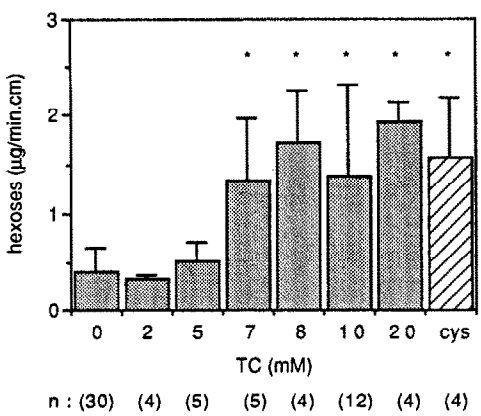

Fig. 4. Intraluminal release of hexoses (glucose equivalents). (Horizontal axis) Concentration of TC in the perfusion solution; n, number of experiment; cys, perfusion with 3% L-cysteine; (vertical axis) intraluminal release of hexoses in $\mu g\ min^{-1}\ cm^{-1}$ (mean value $\pm$ SD); * output of hexoses is significantly higher than perfusions without additive in the perfusion solution ($p < 0.05$; two-sided t-test for unpaired samples).

results of the hexose determinations are depicted in Fig. 4. For TC concentrations ranging from 7 to 20 mM the release of hexoses in the perfusion solution is significantly higher than in solutions without TC. The addition of 3% L-cysteine to the perfusion solution results in the release of hexoses being similar to that for perfusion with micellar concentrations of TC in the medium.

As shown in Fig. 3, the reduction of the viscosity of mucus by TC shows a TC concentration dependency below the CMC of TC. In vivo, the effect of TC on the intraluminal release of hexoses is also related to the concentration of TC: below the CMC, TC does not affect the intraluminal release of hexoses; above the CMC, the release of hexoses is increased, and also independently of the concentration of TC as was observed with the viscosity determinations of the mucus. It is questionable whether the increased in situ release of hexoses at micellar concentrations of TC is the result of a facilitated erosion of the mucous layer because of the reduced viscosity of mucus, or the result of an increased mucus production and secretion. For the further elucidation of this phenomenon, it is necessary to measure the thickness of the mucous layer and to determine the mucus turnover in vivo. If only erosion causes the increased output of hexoses, the mucous layer will decrease in thickness during perfusion. A decreasing stagnant water layer will be reflected in the absorption rate.

Absorption experiments

The observed changes in the mucous structure and hexose output may have consequences for the barrier function of the mucous layer in the absorption of lipophilic drugs. In order to study the relative importance of the barrier function of the mucous layer in the absorption process of the lipophilic drugs DA and KE, perfusion experiments were performed with 10 mM TC or 3% L-cysteine. In Table 3 the results of the absorption experiments with DA and KE with and without 10 mM TC or 3% L-cysteine in the perfusion solution are presented.

The disappearance rates of DA and KE are reduced in the presence of 10 mM TC. As reported before (Poelma et al., 1990), the disap-

TABLE 3

The influence of 10 mM taurocholate and 3% L-cysteine on the absorption of dantrolene (DA) and ketoconazole (KE)

Drug	Perfusion solution	r_{obs} (±SD)	n	CI	f
DA	10 mM TC	0.54±0.07	5	0.45–0.63	0.77
	3% L-cysteine	0.97±0.08	4	0.84–1.10	≈1.0
KE	10 mM TC	0.51±0.18	6	0.32–0.70	0.48
	3% L-cysteine	0.99±0.09	5	0.88–1.10	1.0

$r_{obs} = k_{dis+}/k_{dis-}$; n is the number of experiments; CI gives the 95% confidence interval of r_{obs}; f indicates the fraction of the drug free in solution.

pearance rate of the lipophilic drugs griseofulvin and ketoconazole is decreased in the presence of 10 and 20 mM TC: this reduction is the absorption rate is proportional to a decrease in the fraction of the drug free in solution due to micellar solubilization, and this suggests, that the barrier function of the pre-epithelial diffusion barrier (including the mucus) is not affected by TC. The reduction in the absorption rate of KE in the presence of 10 mM TC ($r_{obs} = 0.51 \pm 0.18$; CI: 0.32–0.70) is not significantly different from the expected absorption profile based on the fraction of drug free in solution ($f = 0.48$). Thus, these results suggest that the absorption barrier for these drugs is not altered by TC. In contrast, for DA the reduction in the disappearance rate cannot fully be explained on the basis of a reduction of the thermodynamically active concentration ($r_{obs} = 0.54 \pm 0.07$; CI: 0.45–0.63 with $f = 0.77$). These results would imply that an increase in the barrier function of the mucous layer by TC contributed to the decrease in the absorption rate of DA. Or, this concept suggests that DA exhibits a different interaction with the mucous layer compared to KE.

In contrast with 10 mM TC, the disappearance rates of DA and KE are not affected by 3% L-cysteine (Table 3: r_{obs} is not significantly different from unity). This is in agreement with the fact that DA and KE are not solubilized by L-cysteine (Table 2). Thus, the thermodynamic activity of the compounds is still unity. Since the disappearance rate of DA and KE is not affected by L-cysteine, this additive supposedly does not affect the overall barrier function of the mucous layer in the intestinal loop, despite the fact that L-cysteine did increase the hexose output (Fig. 4).

One can speculate that a compensatory mechanism restores the barrier function instantaneously, e.g. by the secretion of fresh mucus from the goblet cells as was suggested by Lewin et al. (1979). This would imply that an increased hexose concentration in the lumen and a change in the gel structure in vitro as was observed with TC and L-cysteine are not necessarily related to a change in absorption kinetics of lipophilic drugs.

Conclusions

The results in this report show that both TC and the mucolytic agent L-cysteine are able to alter the rheological properties (viscosity) of native mucus, both in vitro and in situ. For TC, this influence is concentration-dependent: the viscosity-lowering effect in vitro increases with concentration, but reaches a maximum at and above the CMC. In vivo, the effect is also enhanced when the CMC is reached and again to a maximum level.

This strong influence on the mucous layer is without significant consequences for the passive transfer of the lipophilic drugs DA and KE from the intestinal lumen to the serosa, as is shown in perfusion experiments in an isolated intestinal segment. At first sight, the disappearance rate is significantly reduced, but this decrease is proportional — at least for KE — to the fall in thermodynamic activity of the compound due to micellar solubilization. DA and KE are not solubilized by L-cysteine and absorption experiments with L-cysteine reveal that the disappearance rate of these drugs is not affected by L-cysteine. These results indicate that the barrier function of the mucous layer is still intact, despite the influence of the two additives, suggesting that the increased turnover of mucus at the luminal site is neutralized by a compensatory mechanism, which restores the barrier function instantaneously, e.g. by an increased production and secretion of mucus from the goblet cells.

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