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Influence of some surface-active agents on nasal absorption in rabbits

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Summary

The nasal mucosa is a potential site for drug absorption, avoiding problems such as first-pass metabolism and/or gastrointestinal decomposition. The nasal route is especially important for the administration of hormones. However, the aid of absorption enhancers is sometimes required to achieve a high enough bioavailability. Until now, bile salts are the most frequent studied nasal absorption enhancers. The frequent use of bile salts as absorption promotors is limited by their local side-effects. Other, non-bile salt surfactants are currently under research for their efficacy and safety. In this study the effect of 6 different surfactants on the nasal absorption of a model compound (Phenol red) was determined in rabbits. Three bile salts, sodium taurodeoxycholate (TDC), sodium glycodeoxycholate (GDC), sodium deoxycholate (DC)) and 3 other surfactants (Cremophor-EL (CEL), polyoxyethylene-9-lauryl ether (BL-9) and sodium taurodihydrofusidate (STDHF)) were used. The concentration of most of the absorption enhancers in the nasal absorption experiment was 20 mmol/l, for CEL and BL-9 it was 1% (w/v) as their exact molecular weight is unknown. The influence of the absorption enhancers on the phenol red bioavailability was calculated from the serum curves. Phenol red serum concentrations were determined spectrophotometrically. It was found that the combination of Phenol red with the bile salts DC and with the ether-type surfactant BL-9 resulted in a fast and almost complete phenol red absorption, related to the intravenous standard ($F_{DC} = 98 \pm 36\%$ and $F_{BL\text{-}9} = 87 \pm 20\%$). STDHF resulted in a relatively high bioavailability of $F_{STDHF} = 83 \pm 18\%$ but the absorption was slow with a mean residence time (MRT) = 36 ± 6 min. The ester type surfactant CEL had no detectable influence on the nasal absorption; the bioavailability of the phenol red did not differ from the bioavailability of the blank administration $F_{CEL} = 21 \pm 6\%$ and $F_{BLANK} = 22 \pm 11\%$. The ester may be hydrolysed in the nasal mucus, either by enzymes or bacteria present in the mucus layer. Surfactant resistance to these degrading activities may be essential. The described procedure with Phenol red as a model compound proved to be suitable for nasal absorption studies.

Introduction

The nasal route of drug administration is a suitable alternative for drugs or hormones which undergo an extensive first-pass metabolism and/or decomposition in the gastrointestinal tract after oral administration. Until now such drugs are mostly administered parenterally.

For a number of drugs and hormones the efficacy of the nasal route has been described in the literature (Hussain et al., 1980; Rubinstein, 1983; Gordon et al., 1985; Salzman et al., 1985; Andersson, 1986). However, not all drugs which are usually administered parenterally are well absorbed

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by the nasal mucosa. In these cases the presence of absorption enhancers may be necessary to achieve a sufficiently high bioavailability. Several kinds of surface-active agents are used as absorption enhancers.

Initially bile salts proved to be effective but the long-term use in therapy of these substances is limited because of their side effects. Bile salts irritate the nasal mucosa, decrease the ciliary function and may even cause ultrastructural abnormalities as has become clear in animal studies (Hirata et al., 1979; Duchateau et al., 1986).

The nasal absorption of bile salt/drug combinations depends on the kind of bile salt and on the kind of drug. Some authors have reported an increase in the bile salt efficacy with increasing hydrophobicity (Moses et al., 1984). Unfortunately, the more hydrophobic bile salts (dihydroxy bile salts) are more ciliotoxic than the less hydrophobic trihydroxy bile salts (Duchateau et al., 1986).

As an alternative for the bile salts, surface-active agents of several classes are recently proposed and tested for their absorption-promoting efficacy in human and in animal studies (Hirai et al., 1981a and b; Silver et al., 1985).

In this study we want to compare the effects of representative surfactants from different classes on the nasal absorption. The effects of a non-ionic ester, a non-ionic ether and an anionic fusidic acid derivative on the nasal absorption of the model compound Phenol red in rabbits were compared with the effect of some anionic bile salts.

Materials and Methods

Animals

The nasal absorption experiments were performed on rabbits. The average weight of the animals was 3270 ± 420 g (mean $\pm$ S.D., $n = 13$). The rabbits were restrained in wooden boxes during the absorption experiments.

Materials

Phenol red (phenolsulphonphtalein sodium salt, obtained from Sigma, U.S.A.) was used as a model substance to study the influence of the surfactants on nasal absorption. Phenol red was dissolved in methylcellulose gel (2%, ≈ 400 mPa · s) with 20 mM or 1% of one of the surfactants. The pH was adjusted to pH = 7.4.

Six surface-active agents as presented in Table 1 were used as absorption promotors:

The bile salts and the fusidic acid derivative were all dissolved as the sodium salts. Fusidic acid and the cholic acids all have a steroid nucleus but in the cholic acids the A-ring of the steroid moiety is in the *trans*-position whereas the A-ring in the fusidic acid molecule is in *cis*-position.

Bile salts and BL-9 were obtained from Sigma (U.S.A.), CEL was obtained from BASF (F.R.G.) and STDHF was kindly donated by Leo Pharmaceuticals (Denmark). In the absorption experiments the concentration of the surfactant was 20 mM except for CEL and BL-9, which were used in a concentration of 1% (w/v).

TABLE 1

Surfactants used

Surfactant	Abbreviation	Type	K_{HPLC}
Taurodeoxycholate	TDC	bile salt	5.58
Glycodeoxycholate	GDC	bile salt	6.62
Deoxycholate	DC	bile salt	27.05
Cremophor-EL	CEL	ester	–
Poly-oxyethylene-9-lauryl ether	BL-9	ether	38.38
Taurodihydrofusidate	STDHF	fusidic acid derivative	5.67

K_{HPLC} is the retention factor in a reversed-phase HPLC system and reflects the hydrophobicity of the investigated compounds (Armstrong and Carey, 1982).

Study design

Of the viscous solution, 0.2 ml containing 10 mg of phenol red (calculated as the base) and the absorption promotor under investigation, was administered intranasally with the aid of a flexible catheter. Left and right nostril were used alternately in the nasal absorption experiment.

A Phenol red/methylcellulose gel containing 15 mg of phenol red (base), was administered intranasally without a surfactant as a control (blank).

After the intranasal administration the rabbit box was kept in an upright position for 1 min to prevent leakage of the solution out of the nostril. Ca. 30 min before all the administrations the rabbits were lightly anaesthesized with an intramuscular injection of Hypnorm (= 10 mg fluanison + 0.315 mg fentanylcitrate per ml, Duphar, The Netherlands). One injection of 0.25–0.30 ml was sufficient to prevent the sneezing reflex of the rabbits upon insertion of the nasal catheter.

For the i.v. administration 1 ml (= 6 mg) of Phenol red injection (USP XXI) was injected into an ear vein of the opposite ear where the samples were taken.

The oral absorption of Phenol red was studied by administering 30 mg of Phenol red (base), dissolved in 3 ml of methylcellulose gel, directly into the gastric cavity with a catheter.

All the administration forms: i.v. injection, oral and intranasal administrations were given in a random order with at least two weeks between the individual administrations. The different doses were chosen to compensate partly for the differences in absorption, expected from earlier experiments (Duchateau et al., 1986).

Assay

Blood samples were taken from a cannulated ear artery for up to 120 min after the intranasal or i.v. administrations and up to 180 min after the oral administration. The cannula was regularly flushed with a solution of heparin-sodium dissolved in 0.9% saline (500 IU/ml). Contamination of the samples was avoided by discarding the first fraction. Blood samples were centrifuged and the serum layer was stored at −24 °C until analysis.

Phenol red concentrations in the samples were assayed spectrophotometrically in duplicate at 560 nm: 200 µl of the serum sample was deproteinized with 350 µl acetonitrile and 100 µl water (or aqueous standard solution) was added. After centrifugation, 500 µl of the supernatant was added to 200 µl NaOH 0.5 M and the light absorption was measured.

The described analytical method is linear in the concentration range as found in this study (0–30 µg/ml). The limit of detection depends on the instrument accuracy: we accepted an error of 10% at the lowest absorption of $A = 0.010$, equivalent to a concentration in the serum sample of 0.02 µg/ml Phenol red.

Interference of serum components also absorbing at the used wave-length was checked in duplicate by comparing calibration curves in serum and water. No interference could be found. The calibration curve is described mathematically as [phenol red] $= -0.001 + 0.167 \times A$. Deproteinisation with acetonitrile has no influence on the recovery of Phenol red.

Pharmacokinetic analysis and statistics

Pharmacokinetic parameters were calculated assuming a one-compartment model. The absolute bioavailability of phenol red was calculated related to the intravenous administration. Corrections were made for the differences in dose and elimination rate constant. The AUCs were calculated using the trapezium rule, extrapolation to infinity was performed with the term C_{END}/k_{eliv}.

Mean residence times and other parameters of the statistical moment analysis for Phenol red were calculated according to the method of Riegelman and Collier. Corrections in the intranasally administered dose were made by determining the amount of Phenol red dropping out of the nostril.

Student's t-test was used for the comparison of the results. Statistical significance was indicated at a level $\alpha = 0.05$.

Results

Absolute bioavailabilities (F) of Phenol red absorption after intranasal, with and without

TABLE 2

The individual Phenol red bioavailabilities (F_{abs} in %), after intranasal administration with 20 mM of the indicated surfactants and oral administration in rabbits, related to the i.v. injection

Surfactant	STDHF	TDC	GDC	DC	CEL	BL-9	Blank	Oral
1	103	121	86	141	17	99	31	16
2	94	41	112	150	20	82	9	7
3	65	47	102	94	18	115	37	1
4	71	69	35	97	17	85	15	2
5		38	55	51	31	62	16	10
6		115	39	75			11	
7		39	39	79			32	
Mean	83	67	72	98	21	87	22	7
S.D.	18	36	33	36	6	20	11	6

surfactant, and oral administration are presented in Table 2 and Fig. 1.

The serum levels after oral Phenol red administration were very low. A representative example of an oral curve is shown in Fig. 2 together with the curves after intranasal Phenol red administration. For the sake of simplicity not all curves are shown. The Phenol red concentrations tend to increase after ca. 120 min.

The nasal absorption of phenol red with CEL is not significantly different from that after the blank nasal administration. Thus CEL has in this experiment no detectable influence on the nasal absorption ($F_{blank} = 22 \pm 11\%$, $F_{CEL} = 21 \pm 6\%$, mean $\pm$ S.D.). The highest absorptions were found with DC ($F_{DC} = 98 \pm 36$) and BL-9 ($F_{BL\text{-}9} = 87 \pm 20$) as absorption promotors.

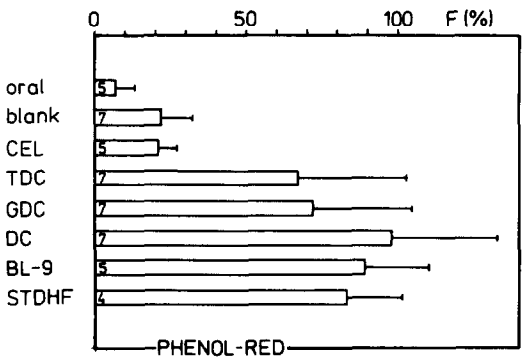

Fig. 1. Absolute bioavailabilities (F) of intranasal Phenol red combined with the different surfactants. See also Table 1. The figures in the bars denote the number of experiments. S.D. values are indicated.

After oral administration of Phenol red the mean residence time (MRT) values are extremely long as could be expected from the serum curves. In Table 3 the individual MRT values are given.

The fast absorption after intranasal phenol red administration with some of the surfactants is clearly expressed in the short MRT values. MRT values of phenol red combined with the bile salts and with BL-9 are not significantly different from the MRT after i.v. administration.

MRT values of the Phenol red/STDHF-, Phenol red/CEL- and Phenol red/blank-administration are significantly longer than after i.v. administration. P-values are < 0.01, < 0.001 and < 0.001, respectively.

These differences in MRT values do not correlate with the differences in F-values. MRT_{STDHF} is significantly longer than the $MRT_{i.v.}$ ($P < 0.01$)

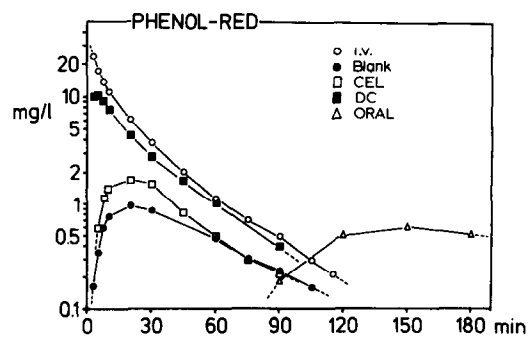

Fig. 2. Representative serum curves obtained in one rabbit after intranasal, oral and i.v. phenol red administration. For the sake of simplicity not all obtained curves are shown.

TABLE 3

The individual MRT-values in minutes after intranasal Phenol red administration in rabbits with 20 mM of the indicated surfactants as absorption enhancers and after oral administration

Surfactant	STDHF	TDC	GDC	DC	CEL	BL-9	blank	oral
	31	32	25	28	41	29	50	159
	29	26	24	25	37	21	30	154
	41	30	31	24	42	27	29	60
	41	39	24	33	39	24	45	157
		25	24	30	38	23	58	59
		33	20	24			36	
		21		22			29	
Mean	36	29	25	27	39	25	40	118
S.D.	6	6	4	4	2	3	12	53

whereas the bioavailability of Phenol red with the STDHF sodium salt is high ($83 \pm 18\%$, $n = 4$).

As could be expected from the bioavailabilities and the serum concentration curves, the MRT-values of Phenol red combined with CEL or the blank administration are significantly longer, $P < 0.001$ and $P < 0.01$, respectively.

Discussion

Due to the very low concentrations of Phenol red in serum, near the limit of detection of the described analytical method, the values resulting from the calculation of the AUC after oral administration have a larger statistical error than the others. This has no important consequences because the resulting bioavailability is very low. It might be possible that for Phenol red an absorption window exists as there was some increase in serum levels after ca. 120 min.

In this study we found an almost complete intranasal absorption of the model compound, Phenol red, if combined with the bile salt DC. The influence of the other two bile salts was not so large. For the TDC- and GDC-phenol red combination the absorption efficacy was 67 ± 36 and $72 \pm 33\%$, respectively. The absorption of the STDHF/Phenol red combination and the combination of BL-9/Phenol red was intermediate, $F_{STDHF} = 83 \pm 18\%$ and $F_{BL\text{-}9} = 87 \pm 20\%$ respectively. Gastrointestinal absorption after a nasal clearing and swallowing of the test substance cannot have contributed to this high bioavailability since the oral bioavailability appeared neglectable.

We found an increase in nasal phenol red absorption in the series of bile salts TDC, GDC and DC. This increase in absorption parallels their increase in hydrophobicity. This was also found for the insulin/bile salt absorption as reported in the literature (Moses et al., 1974). This increase in absorption with the increase of the surfactant hydrophobicity may depend on the drug. It could not be demonstrated for gentamicin combined with TDC, GDC and DC (Duchateau et al., 1986). For deoxycholate gentamicin combinations the pK_a–pH relation results in a diminished solubility and absorption promoting activity of the deoxycholate salts.

The increase in Phenol red absorption with increasing surfactant hydrophobicity is not applicable for all the tested surfactants; BL-9 is very hydrophobic but the absorption is less than the absorption of Phenol red/DC.

The low bioavailability of the combination with CEL, not different from the blank absorption, may be explained by the ester-type of the surfactant. It is likely that the effect of the surfactant is diminished by hydrolysis at the level of the nasal mucosa. Hydrolysis is possible by enzymes present in the mucus layer or by bacteria also present in the mucus. The activity of the other surfactants is apparently not afflicted by hydrolysis.

The effect of the surfactants on the activity of the enzymes present in the nasal mucosa may be responsible for the demonstration by other workers

that insulin is effectively absorbed from the mucosa if combined with a bile salt. It has been shown that bile salts reduce the proteolytic activity of the enzymes and thus the amount of insulin to be absorbed is larger (Hirai et al., 1981a).

Hydrophobicity and resistance of the surfactant against the activity of the enzymes or bacteria may be essential to be effective as an absorption enhancer.

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