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Bile salts and intranasal drug absorption

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Summary

The nasal route has been proven to be very effective for drug absorption. Bioavailability of intranasally administered drugs depends on the structure of the drug and can be as high as 100%. The absorption of drugs with a lower bioavailability can be improved with the aid of absorption promotors such as some surfactants, e.g. bile salts. The intranasal absorption of gentamicin, as a model drug, was studied in rabbits with six different bile salts as absorption promotors (cholate, taurocholate, glycocholate, deoxycholate, taurodeoxycholate and glycodeoxycholate). Without a surfactant, gentamicin is not absorbed by the nasal mucosa. The serum concentrations of gentamicin after intranasal administration were compared with those obtained after an intravenous injection. Concentrations were measured with EMIT. The bioavailability of the intranasal gentamicin in rabbits was related to the hydrophobicity and the pK_a's of the bile salts. Furthermore the effects on ciliated epithelium of the six bile salts, used in the absorption experiment, were studied in an in vitro model. Ciliary activity was studied with a photo-electric method. Bioavailability increased with the increase of the hydrophobicity of the trihydroxy bile salts (cholate, taurocholate and glycocholate). Sodium cholate and sodium taurodeoxycholate were the most active absorption promotors ($F = 41 \pm 16\%$, respectively, $34 \pm 13\%$). The dihydroxy bile salts (deoxycholate, taurodeoxycholate and glycodeoxycholate) showed a decreasing activity in the promotion of gentamicin absorption as the hydrophobicity increases. Depending on the pH/pK_a relation, increasing hydrophobicity results in lower solubility and therefore decreasing activity. Ciliotoxicity of the bile salts increased with increasing hydrophobicity. Dihydroxy bile salts are more toxic than trihydroxy bile salts. At the used concentrations ciliary beat arrested within 30 min. Deoxycholate is extremely ciliotoxic, ciliary arrest occurred within 1 min at a concentration of 5 mmol/l.

Introduction

In recent literature the intranasal route of drug administration for systemic effects has been proven to be very effective (Chien, 1985). Especially for drugs which undergo an extensive first-pass metabolism or are sensitive to gastrointestinal decomposition, the intranasal route may be a suitable alternative. The physicochemical properties of the drug are of importance for the nasal membrane passage, although not all aspects are fully understood at the moment. Both hydrophobic drugs like propranolol and hydrophilic drugs like clofilium tosylate, are reported to be absorbed by the nasal mucosa (Hussain et al., 1980; Su et al., 1984). Thus hydrophobicity seems not to be the only determining factor in nasal drug absorption.

Bile salts (BSs) and other surfactants have been used as early as 1932 to enhance the absorption of peptides (insulin) and drugs across the nasal mucous membrane and across rectal and vaginal mucous membranes (Collens and Goldzieher, 1932; Touitou et al., 1978).

The aminoglycoside gentamicin is not absorbed

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in measurable quantities by the nasal mucosa without a surfactant. The combination of gentamicin and 1% glycocholate in a nasal formulation was effective in achieving therapeutic serum concentrations in humans (Rubinstein, 1983).

BSs are the most widely used surfactants for absorption promotion in nasal solutions. At relatively low concentrations (10–20 mmol/l) they are able to improve the absorption of drugs and peptides (Collens and Goldzieher, 1932; Hirai et al., 1977, 1981; Hirata et al., 1979; Moses et al., 1983; Pontiroli et al., 1983, 1985; Rubinstein, 1983).

Until now, little information is available about the specific requirements of the surfactant molecule structure, and about the local effects of the surfactants on the nasal mucosa.

The aim of this study is to compare the influence of six different BSs on the absorption of gentamicin as a nasal solution in rabbits, and to find a relation between their physicochemical properties and the absorption enhancing efficiency.

Secondly, the effects of the BSs on ciliated epithelium are studied in an in vitro model (Van de Donk et al., 1980).

Materials and Methods

Animals

For the absorption experiments with intranasal gentamicin/BS solutions 5 rabbits were used. The average weight of the animals was 4770 ± 220 g (mean ± S.D.). The rabbits were restrained in a wooden box during the experiments.

Bile salts (BSs)

Six BSs were used in the study:

	Abbreviation	Purity
Sodium tauro-cholate	(TC)	> 98%
Sodium glyco-cholate	(GC)	> 95%
Sodium cholate	(C)	> 95%
Sodium tauro-deoxy-cholate	(TDC)	> 98%
Sodium glyco-deoxy-cholate	(GDC)	> 95%
Sodium deoxy-cholate	(DC)	> 95%

TC, GC and C are trihydroxy BSs and the deoxycholates TDC, GDC and DC are dihydroxy BSs.

BSs were obtained from Sigma (U.S.A.), their purity was checked by means of thin-layer chromatography and in all the absorption experiments the concentration was 20 mmol/l (≈ 1%).

Dose regimen

Gentamicin (25 mg, as sulphate) was administered intranasally in combination with each of the six BSs as a 20 mmol/l solution in a random order. Each rabbit also received an intravenous injection of gentamicin (10 mg, as sulphate) to calculate the absolute bioavailability (F). The gentamicin/BS solutions were prepared in isotonic phosphate buffer, pH 7.4, with 2% methylcellulose (± 400 mPa · s). The viscous solution, 0.5 ml, was administered intranasally in one nostril of the rabbit with the aid of a flexible catheter. To prevent a sneezing reflex the rabbits were slightly anaesthesized with diethylether. Immediately after the administration of the viscous solution the rabbit box was kept in a vertical position for half a minute, to prevent leakage of the solution out of the nostril. A wash out period of at least 3 weeks separated the experiments.

Gentamicin-sulphate was obtained from Sigma (U.S.A.) and the i.v. injections (40 mg/ml) were from Schering Co. (U.S.A.).

Samples and assay

Blood samples (1 ml) were taken out of a cannulated ear artery at 0, 5, 10, 15, 30, 45, 60, 120 and 240 min. The cannula was regularly flushed with a solution of heparin-sodium in 0.9% saline (500 IU/ml). Contamination of the samples with the heparin solution was avoided by discarding the first fraction of the sample. Blood samples were centrifugated and the serum layer was transferred to glass tubes and stored at −24°C until analysis. Gentamicin serum concentrations were determined with an enzyme immunoassay (EMIT, Syva Co., U.S.A.). Heparin has in the used amounts no influence on the the EMIT assay of gentamicin (O'Connell et al., 1984).

Hydrophobicity study

The relative hydrophobicity of the BSs was

determined with a reversed-phase HPLC system (RP-HPLC) (Armstrong and Carey, 1982). Stationary phase: Hypersil-ODS, 5 μm, (Shandon, U.K.), mobile phase: methanol/buffer (pH 5) mixture (75 : 25). Detection at 210 nm.

Ciliotoxicity study

Ciliary activity is one of the most important factors in mucociliary clearance. Recently a good correlation between the mucociliary clearance and the ciliary beat frequency (CBF) in volunteers has been reported (Duchateau et al., 1985). Therefore, the effects of the BSs on the CBF was studied on chicken ciliated embryonal tracheal tissue, using a photo-electric method (Van de Donk et al., 1980) CBF was measured frequently until no more ciliary activity could be observed. The concentration of the BSs was 5 mmol/l for the dihydroxy BSs and 30 mmol/l for the trihydroxy BSs. In the used concentrations, 5, respectively, 30 mmol/l, the ciliotoxicity was comparable for both groups of BSs. All CBF experiments were carried out 6 times. The influence of the BS concentration of CBF was studied with C in a concentration range of 5–80 mmol/l. Ciliotoxicity is presented as the $T_{50\%}$, this is the time at which the CBF has dropped to 50% of the initial frequency, at the used concentrations.

Calculations

Pharmacokinetic parameters were calculated assuming a one-compartment model. Absolute bioavailabilities were calculated from the AUC and the i.v. curve as a reference. The AUCs were calculated using the trapezium rule, extrapolation to infinity was performed with the term C_{END}/k_{el}.

Results

Bioavailability study

The individual bioavailabilities (F) of the gentamicin/BS combinations, compared to the i.v. injection are presented in Table 1.

Combination of gentamicin with DC resulted in very low gentamicin serum concentrations, at the under limit of the EMIT-assay (0.5 μg/ml). The use of these data in the calculation of the k_{el} can lead to erroneous values of the AUC. The highest serum levels of gentamicin were obtained after combination of gentamicin with C or with TDC. Absorption of gentamicin/BS combinations was very fast, time to peak concentration was 15 min or less, except the combination of gentamicin and DC, which gave a slow and irregular intranasal absorption.

TABLE 1

ABSOLUTE BIOAVAILABILITY (F) OF INTRANASAL GENTAMICIN (25 mg) IN COMBINATION WITH 6 DIFFERENT BILE SALTS (BSs) ADMINISTERED TO 5 RABBITS. MEAN AND S.D. ARE ALSO GIVEN.

Rabbit No.	F (%)					
	TC	GC	C	TDC	GDC	DC
1	18	15	45	39	19	20
2	11	30	37	28	12	18
3	15	36	18	20	12	7
4	24	16	45	31	20	5
5	43	45	62	53	14	4
mean	22	28	41	34	15	11
S.D.	13	13	16	13	4	8

Hydrophobicity determination

The results of the BS hydrophobicity determination with the RP-HPLC system are given in Table 2. Results are presented as the retention factor κ. Increasing κ-values reflect increasing hydrophobicity.

In Fig. 1, the F of the gentamicin/BS combinations are plotted against the retention factors of

TABLE 2

RETENTION FACTORS (κ) OF THE 6 BSs USED IN THIS STUDY ON A REVERSED-PHASE HPLC SYTEM. S.D. IS INDICATED, n = 3.

Bile salt	κ	S.D. (n = 3)
TC	0.61	0.01
GC	1.70	0.02
C	3.94	0.03
TDC	1.28	0.02
GDC	3.61	0.04
DC	8.68	0.09

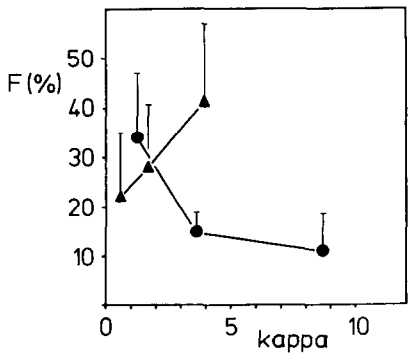

Fig. 1. Absolute bioavailability (F) of intranasal gentamicin/BS combinations in rabbits, plotted against the retention factor on a reversed-phase HPLC system (≈ hydrophobicity). ▲, trihydroxy BSs; ●, dihydroxy BSs.

the BSs. There is a marked increase in F as the hydrophobicity of the BSs increases for the trihydroxy BSs. The opposite trend is perceptible for the dihydroxy BSs. The correlation for F and the κ-value of the BSs is: $r = 0.54$ and $r = -0.68$, for the tri- and dihydroxy BSs, respectively ($P < 0.025$ and $P < 0.005$, respectively, n = 15). The same relation could be found between the cholesterol-solubilizing capacity and F. A positive correlation exists between the F and the cholesterol-solubilizing capacity for the trihydroxy BSs. The cholesterol-solubilizing capacity is expressed as the mole-fraction of cholesterol-monohydrate solubilized in a 100 mmol/l BS solution. The mole-fraction indicates the "power" of the BS to form micelles. The data of the mole fractions are from Armstrong and Carey (1982) (see Table III and Fig. 2).

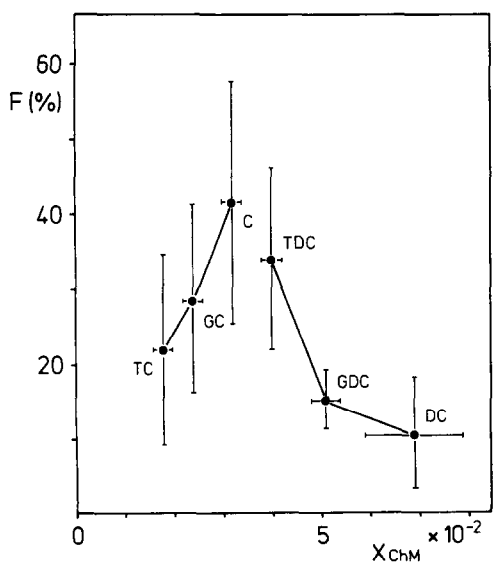

Fig. 2. Absolute bioavailability (F) of intranasal gentamicin/BS combinations in rabbits, plotted against the mole-fraction (X_{ChM}) of cholesterol-monohydrate solubilized by the bile salt. Data of mole-fractions are from the work of Armstrong and Carey (1982).

TABLE 3

MOLE FRACTIONS OF CHOLESTEROL-MONOHYDRATE (X_{ChM}) SOLUBILIZED BY THE BSs. DATA FROM ARMSTRONG AND CAREY (1982)

Bile salt	X_{ChM}	S.D. (n = 3)
TC	0.018	0.002
GC	0.024	0.002
C	0.032	0.002
TDC	0.040	0.002
GDC	0.051	0.051
DC	0.069	0.010

$X_{ChM} = [ChM]/([ChM]+[BS])$

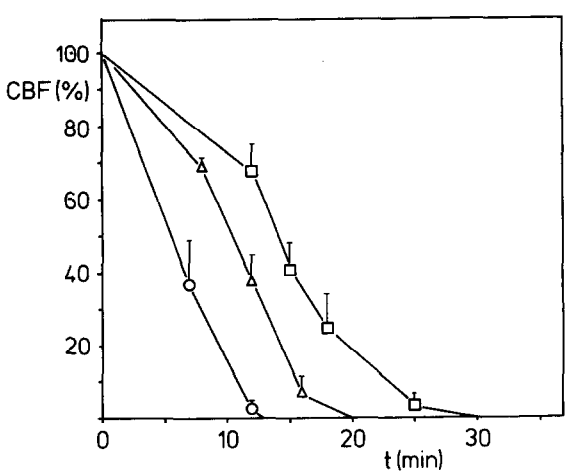

Fig. 3. Time versus frequency plot of trihydroxy BSs at a concentration of 30 mmol/l. S.E.M. is indicated (n = 6). ○, C; △, GC; □, TC.

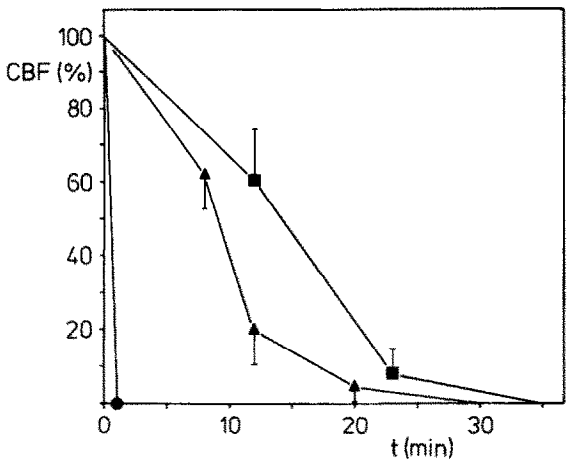

Fig. 4. Time versus frequency plot of dihydroxy BSs at a concentration of 5 mmol/l. S.E.M. is indicated (n = 6). ●, DC; ▲, GDC; ■, TDC.

Ciliotoxicity determination

In Figs. 3 and 4, the in vitro decrease in CBF against time is plotted for the trihydroxy and dihydroxy BSs, respectively. Dihydroxy BSs are more ciliotoxic than trihydroxy BSs. From these curves the $T_{50\%}$ was calculated (Table 4) and plotted against the κ-values (Fig. 5). Ciliotoxicity increases as the hydrophobicity increases for both groups of BSs. The correlation between the κ-value of the BSs and the $T_{50\%}$ of the individual CBF experiments is: $r = -0.88$ and $r = -0.97$ for the tri- and dihydroxy BSs, respectively ($P < 0.005$ for both correlations, n = 18).

In a concentration range from 5 to 80 mmol/l of C a linear relation exists between the logarithm of the $T_{50\%}$ and the concentration ($\log T_{50\%} = -0.02 \times [BS] + 1.36$, $r = -0.98$, $P < 0.005$, n = 10).

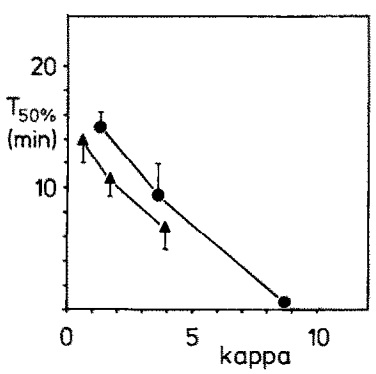

Fig. 5. Mean $T_{50\%}$ values (n = 6) from the ciliotoxicity experiment plotted against the κ-values of the six BSs. ▲, trihydroxy BSs; ●, dihydroxy BSs.

TABLE 4

MEAN TIMES (min) TO REDUCE THE CBF TO 50% OF THE INITIAL FREQUENCY OF 6 BSs. S.D. IS INDICATED, n = 6.

Bile salt	mean $T_{50\%}$ (min)	S.D. (n = 6)
TC	13.9	1.8
GC	10.7	1.3
C	6.8	1.8
TDC	15.0	1.2
GDC	9.4	2.6
DC	0.6	0.2

Discussion

In this study we found the highest F of the combination of gentamicin and C (F = 41%). The other BSs used in combination with gentamicin gave all a lower F, especially the influence of DC on the nasal absorption of gentamicin is low (F = 11%). Insulin administered as an intranasal insulin-DC (1% w/v) aerosol has an absorption efficiency of approximately 10%, compared with the intravenous injection in humans (Moses et al., 1983). In a range of BSs the efficiency was well correlated ($r = 0.95$) with the retention time ($\approx$ hydrophobicity) on a RP-HPLC system (Moses et al., 1984). The studied BSs were in the following order the most effective in the promotion of the intranasal insulin absorption: DC > Cheno-DC > C > Urso-DC. In animal studies no relationship could be found between the BS structure and the intranasal insulin absorption (Hirai et al., 1981). No major difference in insulin absorption, measured as the decrement of plasma glucose, administered with different BSs could be found.

The mechanism of the BSs on intranasal drug absorption is not clear. The influence of surfactants can be at the level of the drug or at the level of the nasal mucous membrane. Solubilizing effects on the drug seem not to be relevant since gentamicin is freely soluble. At the level of the nasal mucosa there are 4 possible modes of action: (1) alteration of the mucus layer (Martin et al., 1978); (2) opening of the tight junctions between the epithelial cells (Inagaki et al., 1985); (3) reversed micelle formation in the membrane; and (4) extraction of membrane components by comicellization (cholesterol, lecithin).

The mucus layer covering the cell surface of the mucosa can be seen as an unstirred layer, acting as a barrier to the diffusion of drug molecules. Anionic and cationic surfactants are able to reduce the mucus viscosity and elasticity, and so the barrier function of the layer (Martin et al., 1978).

It is also possible that BSs can cause the tight junction between the mucosal cells to open. The tight junction opens as a reaction on chemical, electrical or surgical stimuli, making absorption of very large molecules possible ($\leq$ 40,000 MW) (Inagaki et al., 1985).

The formation of reversed micelles, as the third possible mechanism of action, in the membranes of the nasal mucosa cannot completely explain the absorption of gentamicin/BS mixtures. Reversed micelles may function as a kind of large and transient pores in the cell membrane. The reversed micelle theory, however, is not compatible with the predominant hydrophilic properties of the surfactants at physiological pH.

A strong correlation exists between the hydrophobicity and the cholesterol solubilizing capacity of BSs (Armstrong and Carey, 1982). In our experiment no increase in F could be found with increasing hydrophobicity for all the used BSs.

As we did not measure the decrease in mucus viscosity or studied the ultrastructure of the nasal mucosa (tight junction) we cannot exclude the first and second proposed mechanism on the basis of our results.

The results of this study indicate that there is a positive relation between the hydrophobicity and the absorption promotion of gentamicin for the trihydroxy BSs, and a negative relation for the dihydroxy BSs. This is not in keeping with the results of Moses et al. (1984) who did not find an inverse relation in absorption promotion between the tri- and dihydroxy BSs. In contrast to the results of the studies with insulin/BS combinations, we did not find any significant influence of DC on the absorption. The absorption promotion could also depend on the structure of the drug.

The difference between the tri- and dihydroxy BSs may be explained by differences in pH/pK_a partition and the low solubility of the dihydroxy bile acids (not as the sodium salts). The pK_a value of deoxycholate is 6.58, so this is likely to precipitate at a physiological pH or lower. pK_a values of the glycine conjugates are in the range of 4–5, and the pK_a value of the taurine conjugates is not yet known, but are likely to fall between -1.5 and 1.5 (Carey, 1984). The higher the pK_a, the higher the undissociated fraction at physiological pH. In the dihydroxy series this results in an insoluble and therefore inactive fraction, in the series of the trihydroxy BSs this results in a soluble lipophilic and therefore more active fraction. It is known that the solubility of the trihydroxy bile acids is much larger than the solubility of the dihydroxy bile acids (Carey, 1984).

The buffer capacity of the methylcellulose gel and the nasal mucus may be too small to keep the deoxycholates in solution after the addition of the acidifying gentamicin sulphate. Only the undissociated bile acids as far as they are soluble are capable of penetrating the cell membrane. Penetration of the cell membrane is important if the BSs act on the level of the membrane (reversed micelles) or the tight junction.

From the ciliotoxicity study it appeared that dihydroxy BSs are more ciliotoxic than trihydroxy BSs and for both groups ciliotoxicity is well correlated with increasing hydrophobicity. C is the less ciliotoxic and it has the largest effect on the F of gentamicin. DC is the most ciliotoxic BS and has almost no influence on the absorption. Ciliotoxicity after long-term use of BSs should be further studied. In animal studies conjugated BSs caused ultrastructural abnormalities in rat nasal mucosa after prolonged administration (Hirata et al., 1979). The use of surfactants as promotors of intranasal drug absorption must not only depend

on their efficiency, but also on their local side-effects on the nasal mucous membrane. The latter is the more important as frequent dosing is required (insulin).

It can be concluded that the solubility of the trihydroxy compounds and a pK_a value which permits a considerable undissociated fraction at physiological pH are favorable for absorption promotional activity. Sodium cholate best fulfills these requirements.

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