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Oral absorption of lipidic amino acid conjugates

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Summary

A series of unlabelled and radiolabelled lipidic peptide conjugates **2a–2f** were synthesised by reduction of **1a–1f** with $NaBH_4$ and NaB^3H_4, respectively. Initial in vitro experiments using Caco-2 cell cultures were carried out, to predict the in vivo oral absorption of the conjugates. The optimal lipophilicity for oral uptake was determined following oral administration of compounds **2a–2f** to rats. The results suggested that conjugation to lipidic amino acids and peptides is a useful approach to improving the absorption of poorly absorbed drugs. Increasing lipophilicity increased the oral uptake to a certain level, but highly lipophilic compounds showed lower levels of oral uptake.

Introduction

The lipidic amino acids and their homo-oligomers, the lipidic peptides, represent a class of compounds which combine structural features of lipids with those of amino acids (Gibbons et al., 1990) and have potential as a drug delivery system (Toth et al., 1991). Because of their bifunctional nature, the lipidic amino acids and peptides can be chemically conjugated to drugs with a wide variety of functional groups. The linkage between drug and lipidic unit may either be biologically stable (i.e., a new drug is formed) or possess biological or chemical instability (i.e., the conjugate is a pro-drug). In either case, the resulting conjugates possess a high degree of membrane-like character: the permeability coefficient of the conjugated drug moiety may be increased to such an extent that its passage across the hydrophobic membrane of the gastrointestinal (GI) tract is facilitated. The long alkyl side chains may also have the additional effect of affording protection to a labile drug from enzymatic degradation.

Lipidic peptides (with variations in the length and number of alkyl chains) were conjugated to benzoquinolizine acid, and the resulting conjugates and parent acid were subsequently evaluated for oral uptake. Oral uptake was assessed in vitro using the Caco-2 cell culture model. This human adenocarcinoma cell line is widely used in drug and peptide absorption studies. It forms confluent monolayers and differentiates to cells

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with an enterocyte-like morphology under standard cell culture conditions (Chantret et al., 1988). Caco-2 cell cultures permit rapid evaluation of the permeability and metabolism of a drug, the opportunity to study mechanisms of drug absorption under more controlled conditions and the opportunity to perform studies on human cells (Artursson, 1991). The oral uptake and translocation of the conjugates was also studied in vivo, by administering the compounds via gavage to rats.

Materials and Methods

Infrared spectra were recorded with a Perkin Elmer 841 spectrophotometer. Melting points are not given for diastereomeric mixtures. ^{1}H-NMR spectra were obtained on Varian XL-300 and Bruker AM500 instruments operating at fields of 300 and 500 MHz, respectively; chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were run on a VG Analytical ZAB-SE instrument, using fast atom bombardment (FAB) techniques. Reaction progress was monitored by thin-layer chromatography (TLC) on Kieselgel PF_{254} using dichloromethane/methanol 10:1 as the mobile phase. Purification was achieved by TLC using Kieselgel $PF_{254+366}$ (Merck) on 20×20 cm plates of thickness 1.5 mm, or column or flash chromatography through Kieselgel G, (system: dichloromethane/methanol 10:1). Solvents were evaporated under reduced pressure with a rotary evaporator. Infrared spectra gave characteristic NH, carbonyl and aromatic absorbances. Purity of the compounds was determined by TLC and high-pressure liquid chromatography (HPLC). HPLC separation was carried out on a Whatman Partisal 5 RAC silica column. HPLC grade dichlormethane (Aldrich) and methanol (Rathburn) were filtered through a 25 μm membrane filter and degassed with helium flow prior to use. Separation was achieved with a solvent gradient beginning with 0% methanol, increasing constantly to 50% methanol at 15 min and decreasing steadily to 0% methanol from 17 to 20 min at a constant flow of 3 ml min^{-1}. The gradient was effected by two microprocessor-controlled Gilson 302 single-piston pumps. Compounds were detected with a Holochrome UV-Vis detector at 254 nm. Chromatographs were recorded with an LKB 2210 single-channel chart recorder.

*2-[9,10-(Methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl-propamido]octan-1-ol (**2a**)*

Keto-ester **1a** (37 mg, 0.08 mM) was dissolved in dichloromethane (4 ml) and the solution cooled to 0°C. Sodium borohydride (8.6 mg, 0.2 mM) was added and stirred at 0°C for 1 h, methanol (2 drops) was added and the mixture allowed to stir for a further 1 h. The mixture was neutrailsed with acetic acid (1/2 drop), the solvent evaporated in vacuo and the residue treated with 2% sodium hydrogen carbonate (5 ml) and extracted with dichloromethane (3×5 ml), dried ($MgSO_4$) and evaporated in vacuo. Yield: 32.8 mg, 93.2%.

NMR ($CDCl_3$): 6.65 (H,s,C_{11}-H), 6.53 (H,s,C_8-H), 6.25–6.05 (H,3 × d,NH), 5.88 (2H,s,O-CH_2-O), 4.54 (H,m,α-CH), 3.95 (H,m,CH_2-OH), 3.75–3.65 (2H,bd,CH_2-OH), 3.41 (H,m,C_2-H), 3.10 (H,m,C_{11b}-H), 3.01 (H,m,C_4-H), 1.25 (10H,m,5 × CH_2), 0.86 (3H,t,CH_3).

MS *m/z* (%): 469 $[M + Na]^+$(100), 447 $[M + H]^+$ (36), 427 (26), 302 (12), 240 (60), 176 (66).

Anal. $C_{25}H_{38}O_5N_2$ (446.63).

Calcd. C 67.22 H 8.59 O 17.91 N 12.54.

Found C 67.17 H 8.54 O 17.87 N 12.52.

*2-[9,10-(Methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl-propamido]decan-1-ol (**2b**)*

Yield: 34.5 mg, 92.2%.

NMR ($CDCl_3$): 6.65 (H,s,C_{11}-H), 6.53 (H,s,C_8-H), 6.05 (H,m,NH), 5.88 (2H,s,O-CH_2-O), 4.35 (H,m,α-CH), 3.90 (H,m,CH_2-OH), 3.75–3.55 (2H,bd,CH_2-OH), 3.40 (H,m,C_2-H), 3.10 (H,m,C_{11b}-H), 3.01 (H,m,C_4-H), 1.26 (14H,m,7 × CH_2), 0.86 (3H,t,CH_3).

MS *m/z* (%): 497 $[M + Na]^+$(100), 475 $[M + H]^+$ (42), 455 (26), 240 (35), 226 (20), 176 (34), 149 (11).

Anal. $C_{27}H_{42}O_5N_2$ (474.69).

Calcd. C 68.31 H 8.94 O 16.85 N 5.90.

Found C 68.28 H 8.91 O 16.76 N 5.84.

2-[9,10-(Methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl-propamido]tetradecan-1-ol (2c)

Yield: 39.4 mg, 94.1%.

NMR ($CDCl_3$): 6.64 (H,s,C_{11}-H), 6.53 (H,s,C_8-H), 6.45–6.01 (H,3 × d,NH), 5.88 (2H,s,O-CH_2-O), 4.60 (H,m,α-CH), 3.92 (H,m,CH_2-OH), 3.75–3.60 (2H,bd,CH_2-OH), 3.41 (H,m,C_2-H), 3.10 (H,m,C_{11b}-H), 3.01 (H,m,C_4-H), 1.28 (22H,m,11 × CH_2), 0.87 (3H,t,CH_3).

MS *m/z* (%): 553 $[M + Na]^+$(100), 532 $[M + H]^+$ (37), 512 (26), 302(14), 240 (59), 228 (32), 189 (33), 176 (66), 149 (18).

Anal. $C_{31}H_{50}O_5N_2$ (530.81).
Calcd. C 70.14 H 9.51 O 15.07 N 5.27.
Found C 70.12 H 9.45 O 15.04 N 5.24.

α-[9,10-(Methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl-propionyl]-ω-(2-iminodecan-1-ol) [imino(1-octyl-2-oxo-1,2-ethanediyl)] (2d)

Yield: 45.8 mg, 90.1%.

NMR ($CDCl_3$): 6.65 (H,s,C_{11}-H), 6.53 (H,s,C_8-H), 6.25–6.05 (2H,bd,2 × NH), 5.88 (2H,m,O-CH_2-O), 4.54–4.25 (2H,2m,2 × α-CH), 3.90 (H,m,CH_2-OH), 3.75–3.55 (2H,bd,CH_2-OH), 3.40 (H,m,C_2-H), 3.10 (H,m,C_{11b}-H), 3.01 (H,m,C_4-H), 1.26 (28H,m,7 × CH_2), 0.86 (6H,t,2 × CH_3).

MS *m/z* (%): 666 $[M + Na]^+$(84), 644 $[M + H]^+$ (20), 622 (17), 447 (9), 419 (10), 298 (18), 240 (38), 176 (52), 142 (100).

Anal. $C_{37}H_{61}O_6N_3$ (643.98).
Calcd. C 70.14 H 9.51 O 15.07 N 5.27.
Found C 70.12 H 9.45 O 15.04 N 5.24.

α-[9,10-(Methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl-propionyl]-ω-(2-iminotetradecan-1-ol)[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (2e)

Yield: 54.4 mg, 91.3%

NMR ($CDCl_3$): 6.63 (H,s,C_{11}-H), 6.53 (H,s,C_8-H), 6.10–6.00 (2H,bd,2 × NH), 5.88 (2H,s,O-CH_2-O), 4.54–4.25 (2H,2m,2 × α-CH), 3.90 (H,m,CH_2-OH), 3.75–3.55 (2H,bd,CH_2-OH), 3.40 (H,m,C_2-H), 3.10 (H,m,C_{11b}-H), 3.01 (H,m,C_4-H), 1.27 (44H,m,22 × CH_2), 0.86 (6H,t,2 × CH_3).

MS *m/z* (%): 779 $[M + Na]^+$(100), 757 $[M + H]^+$ (31), 739 (17), 302 (22), 242 (24), 200 (52), 176 (69), 149 (15).

Anal. $C_{45}H_{77}O_6N_3$ (756.22).
Calcd. C 71.47 H 10.28 O 12.69 N 5.55.
Found C 71.43 H 10.23 O 12.61 N 5.48.

α-[9,10-(Methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl]propionyl]-ω-(2-iminotetradecan-1-ol)-bis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (2f)

Yield: 71.3 mg, 92.1%.

NMR ($CDCl_3$): 6.66 (H,s,C_{11}-H), 6.55 (H,s,C_8-H), 6.45–6.10 (3H,bd,3 × NH), 5.88 (2H,s,O-CH_2-O), 4.40–4.15 (3H,3m,3 × α-CH), 3.85 (H,m,CH_2-OH), 3.75–3.55 (2H,bd,CH_2-OH), 3.40 (H,m,C_2-H), 3.10 (H,m,C_{11b}-H), 3.01 (H,m,C_4-H), 1.25 (66H,m,33 × CH_2), 0.87 (9H,t,3 × CH_3).

MS *m/z* (%): 1004 $[M + Na]^+$(100), 982 $[M + H]^+$(14), 785 (9), 745 (13), 302 (17), 242 (19), 198 (69), 176 (42).

Anal. $C_{38}H_{59}O_7N_3$ (981.63).
Calcd. C 72.19 H 10.70 O 11.41 N 5.70.
Found C 72.11 H 10.63 O 11.36 N 5.65.

Caco-2 cell experiments

Cells

Caco-2 cells were obtained from the American Type Culture Collection, Rockville, MD. The cells were cultivated on polycarbonate filters (Transwell, Costar, Badhoevedorp, The Netherlands) for transport experiments as described previously (Artursson, 1990). The cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 1% non-essential amino acids, benzylpenicillin (10 IU/ml) and streptomycin (10 μg/ml). Cells of passage 97 were used.

Integrity of the cell monolayers

The integrity of the cell monolayers was tested after 21–22 days in culture by measurement of transepithelial electrical resistance and transport of [^{14}C]mannitol as described previously (Anderberg, 1992). Control monolayers had a transepithelial electrical resistance of ≥ 300 ohm cm^2 and mannitol permeability of $9.6 \pm 0.6 \times 10^{-8}$ cm/s. The integrity of the monolayers was also checked after the transport experiments by measurement of transepithelial electrical resistance.

Transport experiments

The cell monolayers were used in transport experiments after 21–22 days. All transport experiments were performed in Hank's balanced salt solution containing 25 mM Hepes (HBSS). The ^{3}H-labelled benzoquinolizine derivatives (**2a–2f**) were dissolved in DMSO and the solutions were diluted in HBSS to final concentrations of $2.0–4.7 \times 10^{-4}$ M. The final concentration of DMSO was 0.1–0.5%. The solutions were sterile filtered and added to the apical side of the Caco-2 monolayers. At regular intervals (30, 60, 90 and 120 min), the cell culture inserts were transferred rapidly to new basolateral chambers and samples were withdrawn and analyzed in a liquid scintillation counter as described previously (Karlsson and Artursson, 1991). Apparent permeability coefficients (P_{app}) were calculated as described previously (Artursson, 1990). After measurement of the transepithelial resistance the filters were washed rapidly three times with ice-cold HBSS and the filter associated radioactivity was determined (Artursson, 1990).

Statistics

Unpaired two-tailed Student's *t*-test was used to test the statistical significance between two independent means.

Oral dosing

Male Wistar rats (inbred, 200–225 g, 8 weeks old) were housed in metabolic cages to prevent coprophagia, to allow monitoring the growth curves and to enable the collection of urine and faeces. The rats were fed a commercial diet (Maintenance Expanded Rat and Mouse Pellet Diet, Bantin and Kingman, Hull, U.K.) and maintained in an air-conditioned environment at 20°C, under a 12 h light/12 h dark schedule.

A single dose of 4 μmol compound in 1 ml corn oil vehicle was administered to each rat by gavage, using a blunt tipped feeding needle inserted into the stomach. The animals were fasted for 12 h prior to dosing and free access to water was given at all times. Animals were killed 2, 6 and 24 h after dosing. A control group were administered normal saline by gavage.

Blood sample collection

The rats were anaesthetized with halothane and 1 ml of blood was withdrawn from the abdominal aorta. The sample was solubilized in 10 ml Scintran at room temperature for 48 h and by heating at 50°C for 20 min. The samples were decolourised with 0.2 ml H_2O_2 (30%) and evaporated to dryness using N_2 flow at 50°C. The sample was left to redisperse in Optiphase Safe scintillation fluid for 24 h and the radioactivity measured.

Organ sample collection

Stomach, small intestine with mesenteric node, large intestine, liver, kidney and spleen were removed for analysis. The stomach was cut longitudinally, the stomach contents removed and the stomach tissue washed gently with distilled water in an effort to remove any particles that were not absorbed, but were adhering to the surface of the tissue. Intestinal luminal contents were removed by cutting the tissue into 2 cm strips and forcing the contents out with a forceps. The intestinal tissue was also longitudinally dissected and washed to try and clear the tissue of adhering, unabsorbed particles. All dissected organs were washed and weighed. Distilled water was added to the individual samples (roughly 1:4 ratio based on the weight of the sample) and the final volume noted. The samples were homogenised for 5 min. An aliquot part of the homogenate was diluted with Scintran and the sample was left for 48 h at room temperature. An aliquot of the solubilized sample was decolorised with 0.2 ml H_2O_2 (30%) and evaporated to dryness using N_2 flow at 50°C. The samples were redissolved in scintillant for 24 h and the radioactivity measured.

Urine sample collection

The urine was collected from each animal at 12 h intervals and the volume noted. A sample from each 12 h interval was removed, decolorised using 0.2 ml H_2O_2 (30%), diluted with scintillant and the radioactivity measured.

Faeces sample collection

Faeces were collected after 24 h and weighed. 200 ml of water was added to each sample and

the samples were homogenised for 10 min. An aliquot of the homogenate was solubilized in 5 ml of Scintran for 48 h and the samples were placed in an ultrasonic bath for 1 h. An aliquot from each sample was decolorized using 0.2 ml H_2O_2 (30%), scintillant added and the radioactivity measured.

Data calculation

Samples were prepared in triplicate and measured for a period of 2 min on the scintillation counter. Results were corrected for background counts, control counts and dilution factors. Results were expressed as (i) the percentage uptake of the administered dose in the organ or blood sample and (ii) the percentage recovery in the urine and faecal samples.

Results and Discussion

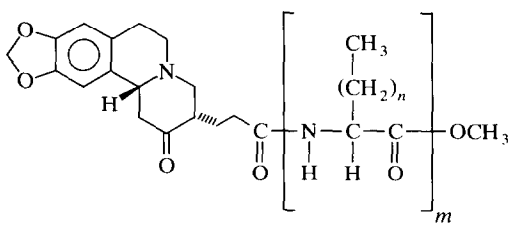
Synthesis of lipophilic benzoquinolizine conjugates

A series of six lipidic peptide conjugates **1a–1f** were prepared by reacting the appropriate lipidic amino acid and peptide methyl esters with the benzoquinolizine acid using literature methods (Toth et al., 1991). Sodium borohydride reduction of the conjugates **1a–1f** gave a series of alcohols **2a–2f** as the major products. A large excess of $NaBH_4$ was used to additionally reduce the terminal ester group to avoid the acid formation in vivo. The configuration of the 2-hydroxyl group was equatorial as anticipated, demonstrated by the chemical shift and axial-axial coupling constants of the proton in position 2. The radiolabelled alcohols **2a*–2f*** were obtained by reduction of the keto function of compounds **1a–1f** with sodium borotrititate in an analogous manner to that described above.

Compounds **2a, 2b** and **2c** represented a series in which the alkyl chain contained six, eight and 12 carbon atoms, respectively, so that the compounds exhibited a gradual increase in lipophilicity. Compounds **2d** and **2e** were more lipophilic, containing two alkyl chains with eight and 12 carbons, respectively; compound **2f** was the most lipophilic, containing three alkyl chains of 12 carbon atoms. The increased lipophilicity was reflected in the increased partitition coefficients of the conjugates (Table 8), which were calculated using an Eluex Version 3.0 program of Compudrug Chemistry Ltd, Budapest, Hungary.

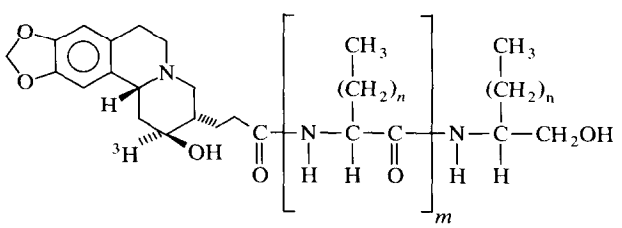
Caco-2 cell experiments

Conjugates **2a–2f** were transported rapidly across the Caco-2 monolayers (Table 1). The apparent permeability coefficients (P_{app}) of conjugates **2d–2f** were approx. 2-fold greater than those of the less lipophilic conjugates **2a–2c** ($p < 0.01$). This increase in transport rate of the conjugates correlated with the increase in the calculated partition coefficients (Table 8). Conjugates **2d–2f**



1	*n*	*m*
a	5	1
b	7	1
c	11	1
d	7	2
e	11	2
f	11	3

Benzoquinolizine lipidic amino acid conjugates **1a–f**



2	*n*	*m*
a	5	0
b	7	0
c	11	0
d	7	1
e	11	1
f	11	2

Tritiated benzoquinolizine lipidic amino acid conjugates **2a–f**

TABLE 1

Apparent permeability coefficients of benzoquinolizine conjugates and transepithelial electrical resistance after the transport experiments in Caco-2 monolayers [a]

Compound	P_{app} (cm/s)($\times 10^7$)	Resistance (ohm cm^2)
–	–	314 ± 16
2a	1.18 ± 0.05	273 ± 29
2b	1.11 ± 0.05	341 ± 26
2c	1.18 ± 0.02	407 ± 42
2d	1.91 ± 0.05	206 ± 42
2e	2.46 ± 0.07	269 ± 78
2f	2.66 ± 0.06	173 ± 3

[a] See Materials and Methods. Mean values ± S.D.

reduced the transepithelial electrical resistance across the monolayers by up to approx. 40% (compound **2f** as compared to controls exposed to buffer only) while no effect was observed for conjugates **2a–2c** (Table 1). This suggests that compounds **2d–2f** increased the permeability of the intestinal epithelium in vitro and enhanced their own absorption.

In general, the absorption of the conjugates to the monolayer/filters was low and only 2–5 % of the radioactivity was found in the filters at the end of the experiments (data not shown). Therefore, no correction of the P_{app} values for filter associated radioactivity was performed.

Absorption of ^{3}H-labelled lipidic conjugates following oral administration

The in vitro cell culture model suggested that conjugation to lipidic peptides could enhance oral absorption, therefore the experiments were extended to investigate in vivo absorption. The ^{3}H-labelled **2a***–**2f*** conjugates were dissolved in a corn oil vehicle and administered by gavage to fasted rats. Animals were killed 2, 6 and 24 h after dosing. Stomach, small intestine with mesenteric node, large intestine, liver, kidney and spleen were removed for analysis. The amount of radiolabel present in the different organs selected, in the blood, urine and faeces (if applicable) was determined. Tables 2–7 and Fig. 1 summarise the results of these experiments.

The radioactivity detectable in the blood and organs following oral administration of the parent benzoquinolizine acid (Toth et al. 1991) was no greater than background.

In contrast, the lipidic conjugates showed significant oral uptake. The extent of oral uptake increased with increasing lipophilicity of the con-

TABLE 2

Oral uptake of compound ***2a***

Organ	% uptake of **2a**		
	1 (3 h)	2 (6 h)	3 (24 h)
Stomach	6.9	1.1	0.3
S.I.	9.8	3.3	0.1
L.I.	0.2	0.9	0.6
Liver	1.8	1.1	0.3
Spleen	0.4	0.1	0.3
Kidney × 2	0.1	0.1	0.3
Organ uptake (%)	19.2	6.6	1.9
Blood	0.2	0.2	6.5
Total uptake (%)	19.4	6.8	8.4

TABLE 3

Oral uptake of compound ***2b***

Organ	% uptake of **2b**		
	1 (3 h)	2 (6 h)	3 (24 h)
Stomach	2.3	0.7	2.1
S.I.	8.6	4.8	3.2
L.I.	0.1	6.9	2.6
Liver	1.5	5.1	7.1
Spleen	0.3	0.7	2.0
Kidney × 2	1.5	0.6	4.0
Organ uptake (%)	14.3	18.8	21.0
Blood	2.0	6.2	10
Total uptake (%)	16.3	25.0	31.0

TABLE 4

Oral uptake of compound ***2c***

Organ	% uptake of **2c**		
	1 (3 h)	2 (6 h)	3 (24 h)
Stomach	4.5	2.1	1.2
S.I.	4.4	6.4	2.2
L.I.	6.3	0.6	1.3
Liver	1.3	3.1	3.6
Spleen	0.2	0.2	3.6
Kidney × 2	0.2	0.5	3.1
Organ uptake (%)	16.9	12.9	15.0
Blood	4.1	22.9	22.1
Total uptake (%)	21.0	35.8	37.1

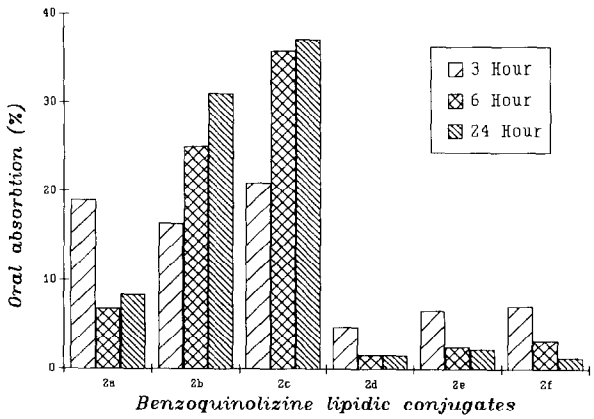


Fig. 1. Oral absorption of benzoquinolizine lipidic conjugates.

jugates, with maximum absorption occurring for conjugate **2c**. The oral uptake decreased for the more highly lipophilic conjugates.

The organ uptake of the least lipophilic conjugate, compound **2a**, decreased from 19.2% (3 h), to 1.9% (24 h); the percentage of the administered dose in the blood increased from 0.2 to 6.5% during this time interval (Table 2; Fig. 1). The increased lipophilicity of compounds **2b** (eight-carbon side chain) and **2c** (12-carbon side chain) increased the oral uptake over the 24 h period with a total uptake of 31% for compound **2b** after 24 h and 37.1% for compound **2c** after 24 h (Tables 3 and 4; Fig. 1).

Further increases in the lipophilicity of the conjugates resulted in a decrease in the oral uptake. Compound **2d** (two alkyl chains with eight carbon atoms) showed a decrease in uptake from 4.75% (3 h) to 1.66% (24 h). Compound **2e** (two alkyl chains with 12 carbon atoms) showed a decrease from 6.61% (3 h) to 2.23% (24 h). Conjugate **2f** (three alkyl chains with 12 carbon atoms) showed a decrease from 3.3% (3 h) to 1.29% (24 h) (Tables 5–7; Fig. 1). In the 24 h experiment the compounds appeared in the urine and the faeces.

Maximum oral uptake of the lipidic system occurred when the log *D* was 6.16. For a water-lipid-water model of intestinal permeability, the permeability should depend on the partition coefficient of the solute. An increased partition coefficient leads to enhanced concentrations within the lipid membrane, greater solute flux and hence greater permeability (Leahy et. al., 1989). For very lipophilic solutes (as in conjugates **2d–2f**), the permeability through the lipid membrane is very high, so that transmembrane flux becomes

TABLE 5

Oral uptake of compound ***2d***

Organ	% uptake of **2d**		
	1 (3 h)	2 (6 h)	3 (24 h)
Stomach	2.50	0.42	0.08
S.I.	1.50	0.52	0.19
L.I.	0.23	0.47	0.31
Liver	0.24	0.16	0.13
Spleen	0.02	0.02	0.05
Kidney×2	0.03	0.06	0.14
Organ uptake (%)	4.52	1.65	0.90
Blood	0.23	–	0.76
Total uptake (%)	4.75	1.65	1.66

TABLE 6

Oral uptake of compound ***2e***

Organ	% uptake of **2e**		
	1 (3 h)	2 (6 h)	3 (24 h)
Stomach	3.84	0.76	1.19
S.I.	2.07	1.08	0.05
L.I.	0.09	0.08	0.07
Liver	0.13	0.23	0.24
Spleen	0.01	0.05	0.06
Kidney×2	0.02	0.14	0.11
Organ uptake (%)	6.16	2.34	1.72
Blood	0.45	0.18	0.51
Total uptake (%)	6.61	2.52	2.23

TABLE 7

Oral uptake of compound ***2f***

Organ	% uptake of **2f**		
	1 (3 h)	2 (6 h)	3 (24 h)
Stomach	1.45	1.02	0.11
S.I.	1.48	1.48	0.09
L.I.	–	0.19	0.14
Liver	0.08	0.07	0.21
Spleen	0.40	0.02	0.22
Kidney×2	0.01	0.04	15 0.06
Organ uptake (%)	3.42	2.82	0.83
Blood	0.08	0.44	0.46
Total uptake (%)	3.50	3.26	1.29

TABLE 8

Calculated partion cefficients of compounds ***2a–2f***

2	Log *D*	Acidic pK_a	Basic pK_a
a	3.04	none	9.6
b	4.08	none	9.6
c	6.16	none	9.6
d	7.17	none	9.6
e	11.32	none	9.6
f	18.07	none	9.6

limited by diffusion across the unstirred water layer. The low water solubility of these highly lipophilic conjugates could therefore be the cause of the lower uptake in vivo. Other factors contributing to the reduced in vivo uptake of conjugates **2d–2f** include the increased tendency of these conjugates to aggregate and precipitate, their higher molecular weight and possible interactions with the mucus layer.

The number and length of alkyl chains in the lipidic conjugating moiety was varied, with a concomitant variation in the partition coefficient of the resulting conjugates. A series of conjugates with increasing partition coefficients was synthesized and the conjugate with optimum lipophilicity for oral uptake was determined. This process of varying the number and length of alkyl chains (and thus the lipophilicity) can be utilized for any drug moiety, so that an optimal lipophilicity for a particular drug molecule can be developed: this demonstrates the versatility of the lipidic amino acid and peptide drug delivery system.

Conclusion

The Caco-2 cell culture model served as a valid initial screening test for the lipidic conjugates, prior to in vivo absorption studies. The results of the oral absorption studies of compounds **2a–2c** were largely in agreement with those of the Caco-2 cell culture experiments: the Caco-2 cell apparent permeability was high and the compounds were taken up orally. These compounds did not show any toxicity.

In the Caco-2 experiments, the highly lipophilic conjugates **2d–2f** showed greatest uptake. This was in contrast to the in vivo situation and may be due to their low water solubility and the other factors discussed.

The results suggested that conjugation to lipidic amino acids and peptides is a useful approach to improving the absorption of poorly absorbed drugs. Increasing the lipophilicity of the conjugates increased their oral absorption, up to a maximum lipophilicty obtained by conjugation with an eight-carbon alkyl chain lipidic peptide. Further increases in the lipophilicity resulted in a decrease in the oral uptake. The optimal lipophilicity of any drug moiety can be determined by conjugation of the drug to lipidic peptides which vary in the constituent length and number of alkyl chains.

References

Anderberg, E.K., Studies on the dissolution of fine particulate practically insoluble drugs and on the effect of surface active enhancers on gastro-intestinal absorption of hydrophilic drugs. Doctorial Thesis, Uppsala University, 1992.

Artursson, P., Epithelial transport of drugs in cell culture: I. A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J. Pharm. Sci.*, 79 (1990) 476–482.

Artursson, P., Cell cultures as models for drug absorption across the intestinal mucosa. *Crit. Rev. Ther. Drug Carrier Syst.*, 8 (1991) 305–330.

Chantret, I., Barbat, A., Dessaulx, E., Brattain, M.G. and Xweibaum, A., Epithelial polarity, villi expression, and enterocyte differentiation of cultured human colon carcinoma cells: a survey of twenty cell lines. *Cancer Res.*, 48 (1988) 1936–1946.

Ewnis, M., Truneh, A., White, J.R. and Pearce, F.L., Inhibition of histamine secretion from mast cells. *Nature*, 289 (1981) 186–187.

Gibbons, W.A., Hughes, R.A., Charalambous, M., Christodoulou, M., Szeto, A., Aulabaugh, A., Mascagni, P. and Toth, I., Lipidic peptides: I. Synthesis, resolution and structural elucidation of lipidic amino acids and their homo- and hetero-oligomers. *Liebigs Ann. Chem.*, (1990) 1175–1183.

Karlsson, J. and Artursson, P., A method for the determination of cellular permeability coefficients and aqueous boundary layer thickness in monolayers of intestinal epithelial (Caco-2) cells grown in permeable filter chambers. *Int. J. Pharm.*, 7 (1991) 55–64.

Leahy, D.E., Lynch, J. and Taylor, D.C., Mechanisms of absorption of small molecules. In Prescott, L.F. and Nimmo, W.S. (Eds), *Novel Drug Delivery and its Therapeutic Application*, Wiley, Chichester, 1989, pp. 33–44.

Toth, I., Hughes, R.A., Munday, M.R., Murphy, C.A., Mascagni, P. and Gibbons, W.A., Lipidic peptides: II. Synthesis, activity and transport of anti-inflammatory benzoquinolizine-lipidic-peptide conjugates. *Int. J. Pharm.*, 68 (1991) 191–198