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Research Paper

The role of molecular physicochemical properties and apolipoproteins in association of drugs with triglyceride-rich lipoproteins: in-silico prediction of uptake by chylomicrons

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Abstract

Objectives The uptake of drugs by chylomicrons is a key element in both intestinal lymphatic transport and postprandial alterations in the disposition profile of lipophilic drugs. The aim of this article was to elucidate the factors that affect this phenomenon.

Methods The degree of association of 22 model lipophilic molecules with rat chylomicrons was assessed and correlated *in silico* with calculated physicochemical properties. The in-silico model was then validated using an external set of molecules. The uptake by chylomicrons was also compared to the association with a marketed artificial emulsion.

Key findings The most important physicochemical property that affects the affinity to chylomicrons was found to be $LogD_{7.4}$; however, a multiparameter model was required to describe properly the uptake process. The in-silico model ($R^2Y = 0.91$, $R^2X = 0.91$ and $Q^2 = 0.82$) that was created using a combination of eight molecular descriptors enabled successful prediction of the affinity of the external set of molecules to chylomicrons. The association with the artificial emulsion was statistically different from the uptake by chylomicrons for four (out of nine) molecules.

Conclusions The association of drugs with chylomicrons is a complex process, which involves the lipophilic core as well as surface apoproteins. The in-silico model based on multiple physicochemical properties of the drugs is able to predict successfully the degree of association with chylomicrons.

Keywords chylomicrons; in-silico model; lipophilic drugs; lipoproteins; physicochemical properties

Introduction

The association of drugs with plasma lipoproteins is a known and widely studied phenomenon.[1,2] However, most research in this area focuses on low-density lipoprotein (LDL) and high-density lipoprotein (HDL), which characterize different pathological dyslipidaemias.[1–6] There is less information concerning the interaction of drugs with larger lipoproteins, having higher triglyceride content (i.e. chylomicrons, chylomicron remnants and VLDL), which appear following consumption of a fat-containing meal.[5,7–13] Chylomicrons, the main component of postprandial hyperlipidaemia, are the largest lipoproteins found in the blood. They are assembled in the enterocytes and then drained via the lymphatic system into the systemic circulation. Chylomicrons and other triglyceride-rich lipoproteins (TRLs) are composed of a lipophilic core (triglycerides with a small amount of cholesteryl ester) and a polar surface (phospholipids, apoproteins and a small amount of free cholesterol).[14–16]

The association of lipophilic drugs with TRLs is an important phenomenon, which is the underlying mechanism of multiple vital pharmacokinetic processes. The location in the body where the uptake of lipophilic molecules by lipoproteins will occur dictates the type of the pharmacokinetic process that will be affected. We have recently shown that intestinal lymphatic transport of lipophilic drugs and postprandial changes in pharmacokinetic and pharmacodynamic profiles are all related to the same event of uptake of lipophilic drug by TRLs.[7,13,17,18] We have also reported a strong linear correlation between intestinal

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lymphatic bioavailability and degree of association of lipophilic compounds with chylomicrons.[17]

Despite the expected influences of the degree of affinity of active molecules to chylomicrons, the data regarding the degree of association of different drugs with chylomicrons in the literature is very limited. Even less is known about the relative role of different physicochemical properties of drugs in the uptake process and the relative role of the lipophilic core and the protein-containing hydrophilic surface of the lipoproteins in this process.

Thus, the goal of this work was to elucidate the factors that affect the degree of association of lipophilic compounds with chylomicrons. This goal was addressed by two specific aims: (1) to elucidate the relative impact of various physicochemical properties of model lipophilic molecules on the degree of association with chylomicrons, and based on this information to develop an in-silico model for prediction of affinity to chylomicrons and (2) to assess the role of surface apoproteins of chylomicrons in the phenomenon of association with lipophilic compounds.

Materials and Methods

Materials

Testosterone, vitamin E, vitamin D_3, benzo[*a*]pyrene, p,p′-DDT, paclitaxel, simvastatin, bifonazole, apomorphine HCl, peanut oil (all from Sigma-Aldrich, Rehovot, Israel), probucol (MP Biomedicals, Inc., Eschwege, Germany) and ciclosporin A (HELM AG, Hamburg, Germany) were all used as received. Diazepam was kindly provided by Taro Pharmaceutical Industries, Ltd (Haifa, Israel). Halofantrine base was prepared from halofantrine HCl tablets (Halfan, GlaxoSmithKline, Marly-le Roi Cedex, France) as previously reported;[19] the purity was confirmed by HPLC (UV and MS detectors). Nineteen synthetic lipophilic cannabinoids (Figure 1) were kindly provided by Pharmos Ltd (Rehovot, Israel). All other chemicals were of analytical reagent grade, and solvents were of HPLC grade.

Production of emulsion of isolated plasma-derived chylomicrons

Male Wistar rats, 300–330 g (Harlan, Israel), were used in this study. The project adhered to the principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and was approved by the Animal Experimentation Ethics Committee of the Hebrew University Hadassah Medical School in Jerusalem. The production of emulsion of isolated plasma-derived chylomicrons was performed similarly to a previously reported procedure.[17] Briefly, rats were fasted overnight with free access to drinking water. Peanut oil (0.5 ml) was administered by gavage 2 h before, and an additional 0.3 ml 1 h before blood collection. Rats were anaesthetized by isoflurane. Terminal blood collection (8–10 ml) from the caudal vena cava was performed. The blood was collected into 12-ml glass tubes, containing 100 μl of EDTA-K_3 10% (w/v), and the plasma was separated immediately by centrifugation (800*g*, 5 min, 15°C). Chylomicrons were separated from 4-ml volumes of plasma by density gradient ultracentrifugation. Phosphate-buffered saline (PBS, pH 7.4) served as standard solutions with density of 1.006 g/ml. Solutions with densities of 1.019 g/ml and 1.063 g/ml were prepared from a solution with a density of 1.006 g/ml by addition of appropriate amounts of KBr. Four millilitres of plasma were adjusted to a density of 1.1 g/ml by KBr (0.57 g) and a density gradient was built in 12-ml polyallomer tubes (Sorvall, Kendro Laboratory Products). Samples were ultracentrifuged (Beckman L5-50B Ultracentrifuge; SW41 rotor, 33 min, 40 000 rev/min, 15°C) and the top 1 ml, representing the chylomicron fraction, was collected using a Pasteur pipette. The chylomicron fractions from several tubes were combined and chylomicron emulsion was diluted with PBS (pH 7.4) to a triglyceride concentration of 100 mg/dl.

Uptake of tested compounds by isolated chylomicrons

The studies of uptake of molecules by isolated chylomicrons (triglyceride concentration 100 mg/dl) were performed as previously published,[17] with an important change that all incubation experiments were carried out at physiological pH using PBS (pH 7.4) as experimental medium for the chylomicron emulsion. Briefly, the stock solutions of most tested compounds (0.1 mg/ml) were prepared in propylene glycol. The stock solutions of extremely lipophilic compounds with $LogD_{7.4} > 9$ were prepared in propylene glycol with 1% ethanol (v/v). An appropriate volume of stock solution of tested drug was added to volumes of 2 ml of chylomicron emulsion (100 mg/dl of triglyceride content) in PBS (pH 7.4) to reach a final molar concentration of 1.75×10^{-6} M. The chylomicron emulsion was incubated with the tested compounds at 37°C for 60 min with constant mixing by magnetic stirrer. After the incubation, the chylomicron emulsion was adjusted to a density of 1.1 g/ml by an appropriate amount of KBr and chylomicrons were separated by density gradient ultracentrifugation. The top 1 ml, representing the chylomicron fraction, was collected using a Pasteur pipette and kept at −70°C until analysis.

Uptake of tested compounds by protein-free artificial emulsion (Intralipid)

The artificial emulsion was diluted by PBS (pH 7.4) to a triglyceride concentration of 100 mg/dl, taking into consideration the amount of free glycerol in the Intralipid emulsion, which was measured similarly to triglyceride concentrations using free glycerol reagent and glycerol standard solution (Sigma-Aldrich, Rehovot, Israel). The incubation of tested compounds with diluted Intralipid and subsequent separation of particles from unbound molecules by density gradient ultracentrifugation was performed in the same way as for natural chylomicrons.

Analytical procedures

The tested compounds were analysed using one of the following systems: (1) Waters 2695 Separation Module HPLC system with Waters 2996 Photodiode Array Detector or with Waters 2475 Multi λ Fluorescence Detector (Waters Corporation, Milford, MA, USA); (2) LC-MS system comprised of Waters pump (600 controller), Waters auto-sampler (717_{plus} Auto-sampler) and Waters Micro-mass ZQ mass

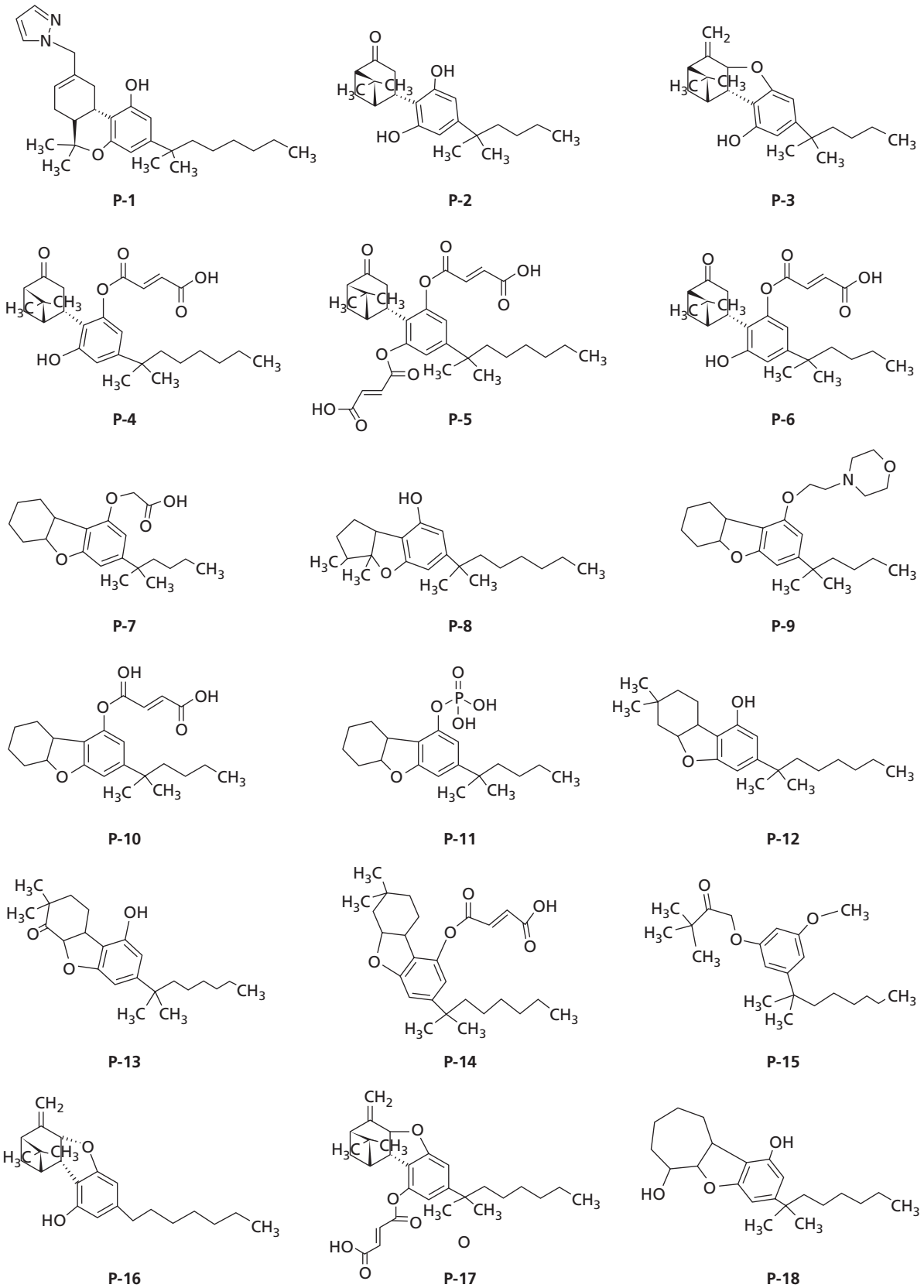

Figure 1 Chemical structure of model synthetic cannabinoids used in this study.

spectrometer (Waters Co., Milford, MA, USA); (3) Hewlett-Packard, HP-6890 series GC system.

The analysis of diazepam, testosterone, probucol, benz[a]-pyrene, vitamin D_3, vitamin E, halofantrine, DDT and bifonazole in chylomicrons or Intralipid samples was performed as previously published.[17] The analysis of simvastatin was performed as previously reported,[20] using testosterone as an internal standard.

The conditions for determination of other compounds used in this study are summarized in Table 1. The calibration curves of all tested compounds were linear ($r^2 > 0.99$) from 0 up to 3.5×10^{-6} M (maximal possible concentration that corresponds to 100% association with chylomicrons). The inter- and intra-day coefficients of variation were below 3%.

Development of in-silico model

To examine the role of physicochemical properties in the association of drugs with chylomicrons, 22 model lipophilic molecules (Table 2) were screened empirically for their degree of uptake by plasma-derived isolated chylomicrons at pH 7.4. Fifteen molecules from this list were novel lipophilic synthetic bicyclic and tricyclic cannabinoids analogous with proven or suspected neuroprotective or analgesic activity provided by Pharmos Ltd (Rehovot, Israel) (Figure 1).

The physicochemical properties of the tested compounds were calculated using ACD/Labs Version 10 (Advanced Chemistry Development Inc., Toronto, Canada). The examination of relative importance and direction of influence of different physicochemical parameters in the association of drugs with chylomicrons and analysis of predictive power of multiple partial least squares projections to latent structures (PLS) models, which comprised different combinations of molecular descriptors, was performed using SIMCA-P version 11.5 (Umetrics, Sweden).

The performance of this established in-silico model was corroborated using an external set of eight molecules that included three additional synthetic cannabinoids (Table 2).

Statistical analysis

The data in this paper is presented as mean ± SD, if not specified otherwise. Difference between affinities of model molecules to chylomicrons was assessed for significance using one-way analysis of variance test (see Figure 2). Differences between association of model molecules with

Table 1 Summary of conditions of analytical procedures

Compound	Sample treatment method	Detection	Column	Mobile phase[a]	Flow (ml/min)	Internal standard
P-1	A	MS, 437 m/z	3, 30°C	Water–AN–MeOH (20 : 70 : 10)	0.2	P-2
P-2	A	MS, 345 m/z	3, 30°C	Water–AN (40 : 60)	0.3	P-1
P-3	A	MS, 341 m/z	3, 30°C	Water –AN–MeOH (15 : 80 : 5)	0.3	P-5
P-4	B	MS, 471 m/z	3, 30°C	Water–AN–MeOH (35 : 65)	0.3	P-6
P-5	B	MS, 569 m/z	3, 30°C	Water–AN–MeOH (80 : 20)	0.25	P-16
P-6	B	MS, 443 m/z	3, 30°C	Water–AN–MeOH (40 : 60)	0.3	P-4
P-7	B	UV, 211 nm	1, 50°C	Buf. Ammon. Acet. 0.1 M, pH 3.5–AN (25 : 75)	1.2	P-10
P-8	A	UV, 211 nm	2, 45°C	Water–AN (20 : 80)	1.2	P-12
P-9	A	UV, 211 nm	2, 50°C	Water–AN (25 : 75)	1.2	P-15
P-10	B	UV, 211 nm	1, 50°C	Buf. Ammon. Acet. 0.1 M, pH 3.5–AN (25 : 75)	1.2	P-7
P-11	B	FL, ex. 275 em. 300	2, 50°C	Buf. Ammon. Acet. 0.01 M, pH 3.5–MeOH (25 : 75)	0.8	None
P-12	A	UV, 211 nm	2, 45°C	Water–AN (20 : 80)	1.2	P-8
P-13	A	MS, 359 m/z	3, 30°C	Water–AN–MeOH (15 : 80 : 5)	0.3	P-16
P-14	B	443 m/z	3, 30°C	Water–AN–MeOH (15 : 80 : 5)	0.3	P-17
P-15	A	UV, 211 nm	1, 50°C	Water–AN (25 : 75)	2	P-12
P-16	A	MS, 369 m/z	3, 30°C	Water–AN–MeOH (15: 80 : 5)	0.3	P-5
P-17	B	MS, 467 m/z	3, 30°C	Water–AN–MeOH (15 : 80 : 5)	0.3	P-14
P-18	C	MS, 407 m/z	5, 220–490°C	Helium	1.0	P-1
Apomorphine	A	FL, ex. 270 em. 450	4, 40°C	Buf. Acet. 0. 1 M, pH 4.6–AN (55 : 45)	1.2	Propranolol
Amphotericin B	B	UV, 405 nm	1, 40°C	Buf. Phosph. 0.01 M, pH 3.5–MeOH (20 : 80) 0–3 3 min, (5 : 95) 7–10 10 min, (20 : 80) 14–17 min	1.5	Benz[a]-pyrene

Sample treatment method A: Following addition of 600 μl of THF to 200-μl volumes of chylomicron emulsion or Intralipid, the mixture was vortexed for 1 min. After extraction by 3 ml of ethyl acetate for 1 min and centrifugation (1050*g*, 7 min), the upper organic layer was decanted to a fresh test tube and evaporated to dryness by vacuum evaporator. The residue was reconstituted with 100 μl of methanol. Seventy microlitres were injected into HPLC system for UV and FL methods of detection, and 20 μl for MS methods of detection. *Sample treatment method B:* The same as method A with acidification of the sample by 100 μl of HCL 0.1 M before addition of THF. *Sample treatment method C* : Extraction from 100 ml of sample by solid-phase extraction on SPE cartridges: Varian, Bond Elut Certify, 10CC/130 mg, CN 1211-3050 followed by derivatization with bis(trimethylsilyl) trifluoroacetamide with 1% chlorotrimethylsilane.

Column 1, LiChrospher RP-18 (5 μm), 250 mm × 4 mm; Column 2, XTerra RP-18 (3.5 μm), 250 mm × 4.6 mm; Column 3, XTerra MS RP-18 (3.5 μm), 100 mm × 2.1 mm; Column 4, BDS Hypersil Cyano (5 μm), 250 mm × 4.6 mm; Column 5, HP-5-MS, 15 m × 0.25 mm, 0.25 mm film thickness. AN, acetonitrile; MeOH, methanol. [a]In LC-MS detection methods the mobile phase also includes 0.05% trifluoroacetic acid and 0.1% formic acid.

Table 2 Physicochemical properties of model molecules

Molecules used for in-silico model development	LogP	$LogD_{7.4}$	PSA	FRB	H donors	H acceptors	Molar volume (cm^3)	Density (g/cm^3)
P-1	8.5	8.5	47.3	9	1	4	394.6	1.11
P-2	5.15	5.15	57.5	7	2	3	317.4	1.09
P-3	7.47	7.47	29.5	5	1	2	312.2	1.09
P-4	6.55	2.91	100.9	12	2	6	410.8	1.15
P-5	6.54	1.79	144.3	15	2	9	471.3	1.21
P-6	5.48	1.85	100.9	10	2	6	377.8	1.17
P-7	5.97	2.33	55.8	7	1	4	312.9	1.11
P-8	7.82	7.82	29.5	7	1	2	326.2	1.01
P-9	6.12	6.03	30.9	8	0	4	382.8	1.05
P-10	6.4	2.78	72.8	8	1	5	336.6	1.15
P-11	4.93	0.61	85.8	6	2	5	304.4	1.21
P-12	8.45	8.44	29.5	7	1	2	347.6	0.99
P-13	6.62	6.62	46.5	7	1	3	344.1	1.04
P-14	8.5	4.87	72.8	10	1	5	408.1	1.08
P-15	5.99	5.99	46.5	11	1	3	340.8	0.98
Diazepam	2.91	2.91	32.7	1	0	3	225.9	1.26
DDT	5.92	5.92	0	2	0	0	244.2	1.45
Benzpyrene	6.4	6.4	0	0	0	0	196.1	1.29
Halofantrine	8.72	6.74	23.5	11	1	2	402.0	1.24
Probucol	10.27	10.27	91.6	10	2	2	480.5	1.08
Vitamin D	9.72	9.72	20.2	7	1	1	396.9	0.97
Vitamin E	11.9	11.9	29.5	13	1	2	462.8	0.93
External Set								
Testosterone	3.48	3.48	37.3	1	1	2	257.0	1.12
P-16	8.53	8.53	29.5	7	1	2	344.5	1.07
P-17	8.58	4.96	72.8	10	1	5	401.99	1.16
P-18	6.35	6.35	49.7	8	2	3	323.9	1.07
Apomorphine	3.05	2.51	43.7	2	2	3	205.6	1.3
Amphotericin B	1.16	−1.41	319.6	14	13	18	689.4	1.34
Bifonazole	4.84	4.78	17.8	4	0	2	288.1	1.08
Simvastatin	4.42	4.42	72.8	8	1	5	376.5	1.11

PSA, polar surface area; FRB, freely rotatable bonds.

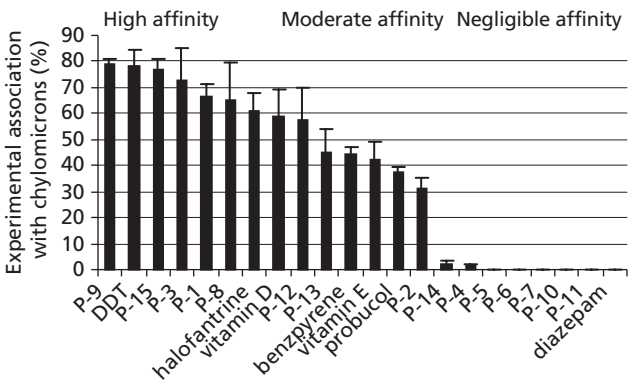

Figure 2 Experimental association with chylomicrons of 22 model molecules used in the development of in-silico model for prediction of the degree of uptake of drugs by chylomicrons. Data are presented as mean ± SD. $P < 0.0001$, one-way analysis of variance test.

natural chylomicrons versus artificial emulsion were assessed for significance using unpaired Student's *t* -test (see Figure 4a). $P < 0.05$ was considered statistically significant.

Results

Experimental association of model molecules with isolated rat chylomicrons

The experimental affinity of 22 model molecules with plasma-derived isolated rat chylomicrons is shown in Figure 2. It can be seen that the model molecules demonstrate a wide range of degree of uptake by lipoproteins, which can be roughly divided into the areas of zero or negligible affinity, moderate affinity and high affinity.

Physicochemical properties and their correlation with the degree of association of model molecules with chylomicrons

The list of ACD/Lab derived physicochemical properties of 22 model lipophilic molecules used for the development of an in-silico model, as well as of 8 molecules used as an external set, are summarized in Table 2. It can be seen that although most molecules in the development set are structurally similar (which could be one of the potential limitations of the proposed in-silico model), this data set presents a relatively wide range of each physicochemical property, including

lipophilicity. The list of correlation coefficients of experimental uptake by chylomicrons with each molecular descriptor listed in Table 2 is as follows: with $LogD_{7.4}$ (the distribution coefficient at pH 7.4), $R^2 = 0.4973$; with polar surface area (PSA), $R^2 = 0.5341$; with number of H-donors, $R^2 = 03061$; with number of H-acceptors, $R^2 = 0.4475$; with freely rotatable bonds (FRB), $R^2 = 0.0369$; with LogP (the partitition coefficient), $R^2 = 0.1795$ and with molecular density, $R^2 = 0.0341$. It can be clearly seen from this list that no one single physicochemical parameter provides a good correlation with the degree of association of model lipophilic compounds with chylomicrons. Interestingly, when compounds with $LogD_{7.4}$ above 9 (probucol, vitamin D_3 and vitamin E) are removed from the data set, superior linear correlation is obtained between affinity to chylomicrons and $LogD_{7.4}$ ($R^2 = 0.7504$ vs $R^2 = 0.4973$). It seems that for compounds with $LogD_{7.4}$ values above 9 there is no additional increase in degree of association with chylomicrons. Most likely, at some stage, the extremely high lipophilicity counteracts with the degree of uptake by chylomicrons, probably due to polar barrier of phospholipids and apoproteins on the surface of lipoproteins. Therefore, the correlations between the empirical data and the in-silico prediction have been performed without these three compounds, bearing in mind that the developed linear models probably will be less accurate for rare cases of drugs with $LogD_{7.4}$ values above 9. When extremely lipophilic compounds with $LogD_{7.4}$ values above 9 are removed from the data set, $LogD_{7.4}$ has the strongest correlation with affinity to chylomicrons at physiological pH than any other physicochemical property, including LogP.

Development of an in-silico model for prediction of affinity of compounds to chylomicrons

To assess the relationship between multiple combinations of physicochemical properties and experimental association with chylomicrons, PLS models[21–24] were developed using SIMCA-P software. The effect of each descriptor was tested by variable importance on projection (VIP). The physicochemical properties with lower VIP values were excluded from the list and the resultant effect on the model was reviewed by cross-validated correlation coefficient (Q^2). If the exclusion of the descriptor induced significant reduction in Q^2 or R^2Y, the physicochemical property was included again in the model. The physicochemical properties that were not included in the model (according to this principle) are molecular weight (MW), predicted water solubility, polarizability, surface tension, index of refraction, parachor, molar refraction and 'rule of 5'. Instead of LogP, the mathematical difference between LogP and $LogD_{7.4}$ was used in the development of the in-silico model since it provided higher predictive power. This difference describes the degree of ionization of the tested molecules at physiological pH (i.e. $\log(1 + 10^{7.4-pKa})$ for acids and $\log(1 + 10^{pKa-7.4})$ for bases.[25]

LogP itself, although found initially to be a relatively important property, was not included in the final model, since better, or the same, Q^2 or R^2Y could be achieved without this descriptor. In addition, LogP is mathematically interrelated with $LogD_{7.4}$ and with LogP – $LogD_{7.4}$. Interestingly, Holm and Hoelst,[26] in the in-silico PLS model for prediction of intestinal lymphatic transfer, also did not use the LogP descriptor. This model had higher predictive power than traditionally used LogP values, and thus was a relatively significant improvement to the prediction method used before. However, the data set of lymphatic transport of model molecules was highly limited due to scarce available information in the literature on the lymphatic bioavailability of lipophilic compounds. In the current work we have chosen to rely only on experimental data that was obtained in our laboratory, which undoubtedly reduced the variability of information.

The descriptors included in the final in-silico model were $LogD_{7.4}$, LogP – $LogD_{7.4}$, PSA, H-acceptors, H-donors, molar volume, FRB and density. Before analysis, the LOGIT (LOG_{10} (X/1 – X)) transformation was applied to the experimental data of the degree of association with chylomicrons as a part of the model establishment. Using the above setup, a final PLS model was generated with $R^2Y = 0.91$, $R^2X = 0.90$ and $Q^2 = 0.83$, which means that this model explains 91% of the Y-variation, 90% of the X-variation and predicts 83% of the Y-variation (predictive power). This model output is superior to correlation of any single descriptor with experimental affinity to chylomicrons. The plot of experimental association with chylomicrons of 19 model molecules used in the final model versus predicted values generated by this model is shown in Figure 3a. The

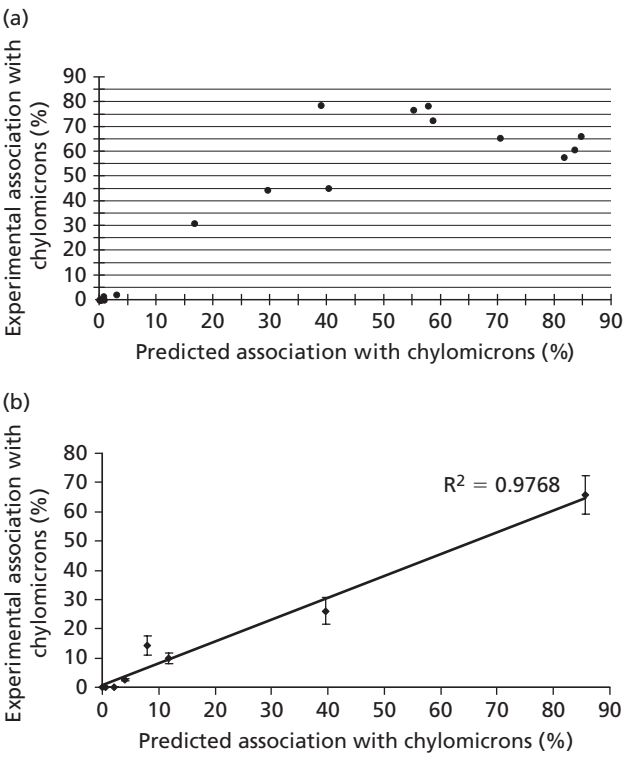

Figure 3 (a) Experimental versus predicted association with chylomicrons of molecules used in the development of the PLS in-silico model. (b) Correlation of experimental uptake by chylomicrons with predicted affinity of external set used in the validation of the developed in-silico model for prediction of degree of association of drugs with chylomicrons. Data are presented as mean ± SD.

Table 3 PLS model derived variable importance on projection (VIP) for each molecular descriptor and multivariate linear regression coefficients

Physicochemical property	VIP	Unscaled regression coefficients
$LogD_{7.4}$	1.43	0.299879
$LogP - LogD_{7.4}$	1.19	–0.238127
PSA	1.07	–0.00855215
H-acceptors	1.04	–0.184359
FRB	0.79	0.0805226
Density	0.76	1.45337
Molar volume	0.76	0.00545912
H-donors	0.74	0.0823094
Constant		–5.24138

PSA, polar surface area; FRB, freely rotatable bonds.

variable importances on projection demonstrating the relative importance of each descriptor in the developed model are shown in Table 3. The unscaled coefficients and a constant of the developed PLS model are shown in Table 3. These coefficients allow description of the developed model as a multivariate regression equation, which make it possible to perform future predictions even without the use of SIMCA-P software (however, one should keep in mind that if these unscaled regression coefficients of the model are used for predictions instead of SIMCA-P software, the back conversion of predicted values from LOGIT transformation should be done manually).

Validation of an in-silico model by an external set of model molecules

The performance of the newly developed in-silico model was tested by eight additional lipophilic molecules that were not used in the establishment of the PLS model itself (Table 2). The linear correlation between the experimental and predicted (using SIMCA-P prediction function) by in-silico model affinity to chylomicrons in the external set ($R^2 = 0.98$) is shown in Figure 3b. This linear correlation is even superior to the output of the developed in-silico model (Figure 3a), which, however, could be explained by a smaller sample size in the external set.

Association of lipophilic molecules with protein-free artificial emulsion versus uptake by natural rat chylomicrons

To elucidate the potential role of surface proteins in the degree of uptake of lipophilic compounds by TRLs, the additional set of association of nine lipophilic model molecules with artificial emulsion (Intralipid) (not having apoproteins on their surface) was compared with the uptake by natural chylomicrons.

The degree of association of nine model lipophilic molecules with natural chylomicrons and protein-free artificial emulsion (Intralipid) is shown in Figure 4a. It can be clearly seen that the compounds that do not tend to associate with chylomicrons (i.e. diazepam, testosterone and ciclosporin) are also not taken up by the artificial emulsion. For four model compounds (probucol, vitamin E, benz[a]pyrene and DDT) the association with artificial emulsion was statistically different from association with natural rat chylomicrons.

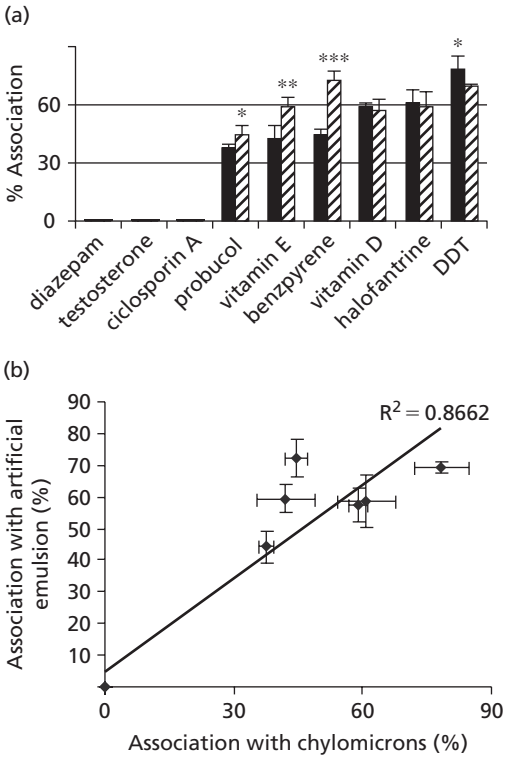

Figure 4 (a) Association of model lipophilic molecules with artificial protein-free emulsion (diluted Intralipid). Black columns, natural chylomicrons; hatched columns, artificial emulsion. Data are presented as mean ± SD. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ natural chylomicrons vs artificial emulsion. (b) Correlation of affinity of model molecules to artificial protein-free emulsion with uptake by natural plasma-derived rat chylomicrons.

Discussion

Experimental association of model molecules with isolated rat chylomicrons

We found that the 22 model molecules studied demonstrated a wide range of degree of uptake by lipoproteins (Figure 2). It was previously shown that compounds with high affinity to chylomicrons will have extensive intestinal lymphatic transport and pronounced changes in disposition and pharmacological activity following a high-fat meal.[12,13,18] On the other hand, compounds with zero, or negligible, association with chylomicrons will have neither intestinal lymphatic transport nor measurable changes in disposition profile following consumption of fat-containing food. It is unlikely that compounds that associate moderately with chylomicrons will have a high component of intestinal lymphatic transport or prominent changes in disposition following a high-fat meal. However, it was shown[18] that such compounds may still produce extremely high concentrations in the lymphatic fluid following oral administration with long-chain triglyceride vehicle, which makes them suitable for targeting to the lymphatic system.

Development and validation of an in-silico model for prediction of affinity of compounds to chylomicrons

It can be discerned from our data that the most important physicochemical properties for the degree of association with chylomicrons in the final PLS model were $LogD_{7.4,}$ LogP – $LogD_{7.4}$, PSA and number of H-acceptors. This observation is extremely important, since previously only LogP and solubility in triglyceride were thought to be important in the association of lipophilic compounds with chylomicrons and in related pharmacokinetic processes. For example, it was proposed that parameters responsible for significant intestinal lymphatic transport of lipophilic molecules (the process that is largely governed by the degree of association with chylomicrons) are LogP > 5 and solubility in triglyceride > 50 mg/ml.[27] However, additional studies have shown that these parameters do not always predict successfully the degree of intestinal lymphatic transport.[17,28–30] Now, it becomes clear that association with chylomicrons is a complex process that cannot be described accurately by a single descriptor or a pair of physicochemical properties, and a combination of multiple descriptors is needed to accurately describe this phenomenon.

The results demonstrate that the proposed in-silico model can be used for the prediction of the degree of affinity of drugs to chylomicrons even before the synthesis of new molecules. It should be noted that the described in-silico model is based on calculated molecular descriptors. Since calculated parameters may considerably differ from experimental values, we recommend using calculated descriptors (preferably by the same software used in this study) when the described model is utilized for predictions. It should be noted also that the described model is developed based on the interaction of lipophilic compounds with rat chylomicrons. However, it is conceivable that it will predict accurately the association of lipophilic drugs with human chylomicrons as well, since rat and human chylomicrons are very similar in their composition (i.e. triglyceride, phospholipid, cholesterol and protein content).[31,32]

Association of lipophilic molecules with protein-free artificial emulsion versus uptake by natural rat chylomicrons

Our findings further corroborate the hypothesis that the association of lipophilic compounds with chylomicrons is a process more complicated than simple partition between water and lipophilic core of lipoproteins. It seems that surface apoproteins play a certain role in the process of uptake at least for some of the molecules. However, there is a relatively good linear correlation ($R^2 = 0.86$) between the uptake by artificial emulsion and natural chylomicrons (Figure 4b). Thus, it is possible to use this in-vitro technique (association with artificial emulsion) as an alternative method for the prediction of the degree of association with chylomicrons, as it does not require any animal involvement. The advantage of this method over the in-silico prediction is that affinity of extremely lipophilic compounds may be also successfully predicted, whereas the main disadvantage is the need for empirical experiments.

Conclusions

The uptake of active molecules by chylomicrons and other TRLs is an important phenomenon that promotes the intestinal lymphatic transport and postprandial changes in the disposition profile of lipophilic drugs. The association of drugs with TRLs is a complex process, which involves the impact of the lipophilic core as well as of surface apoproteins, and thus cannot be described by single molecular descriptors. The important physicochemical properties that affect the degree of association in physiological conditions are $LogD_{7.4}$, degree of ionization (LogP – LogD7.4), PSA, number of H-acceptors and H-donors, density, molar volume and FRB. The combination of these physicochemical properties describes more accurately the process of uptake of molecules by chylomicrons than any single descriptor.

The association of tested drug with natural chylomicrons *ex vivo* at pH 7.4 is the most accurate way to assess the affinity of the compound to chylomicrons. However, when a larger set of molecules is to be screened or if the use of laboratory animals is undesirable, the degree of association with artificial emulsion may provide a satisfactory level of prediction. The important advantage of the proposed in-silico model is that the predicted value can be determined even if the molecule has not been synthesized yet.

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Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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