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Note

Successful in silico predicting of intestinal lymphatic transfer

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Abstract

The possibility of developing a quantitative relationship between molecular structure and lymphatic transfer of lipophilic compounds co-administered with a long-chain triglyceride vehicle was examined. Molecular descriptors were calculated using the computer program VolSurf, and lymphatic transfer data were derived from the literature. A significant structure–property relationship was established using partial least squares, projection to latent structures statistics (PLS). R^2X was 0.77, R^2Y was 0.83 and the prediction power of Q^2 was 0.73 in the two-component PLS model. A number of descriptors contributed to the prediction leading to a complex model, but the prediction power was improved with the PLS model when compared to the frequently used method by relating log *P* values (LogKow) with lymphatic transfer.
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Keywords: Lymph; Lipid; Triglyceride; In silico prediction; VolSurf; PLS

The introduction of combinatorial chemistry and in vitro activity high throughput screening methods in drug discovery has accelerated the number of compounds investigated in industrial drug discovery. These compounds are, however, frequently characterized as low aqueous soluble, which, in combination with other, both physicochemical, pharmacokinetic and biopharmaceutical, properties, can lead to low and variable bioavailability (Lipinski et al., 1997). Based on these intrinsic properties of the molecules potent compounds may fail to progress into clinical studies due to problems achieving a desirable pharmacokinetic profile (Andrews et al., 2000).

An increasingly popular approach to overcome the low oral bioavailability is the incorporation of the active lipophilic component into lipid vehicles such as oil solutions and self-emulsifying drug delivery systems (Humberstone and Charman, 1997), as demonstrated by the commercial success of lipid-based formulations of cyclosporine A (Sandimmun Neoral™), saquinavir (Fortovase) and ritonavir (Norvir). The increased application of lipids has lead to further exploration of the intestinal lymphatic system as some of these excipients facilitates transfer of lipophilic drugs into this absorption pathway (Caliph et al., 2000).

The lymphatic transfer mechanism of lipophilic molecules from the intestine is not clear, but the requirement for co-administration with a long-chain triglyceride is well documented (recently reviewed by O'Driscoll, 2002). Ichihashi et al. (1994) therefore examined the relationship between the partition ratio of various drugs absorbed into either the lymphatic systemic or the systemic circulation when co-administered with a long-chain triglyceride to lymph cannulated rats. A relationship between the lipophilicity and the logarithm of the distribution was

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Table 1
Lymphatic transport data for the compounds co-administered with a long-chain triglyceride used for the predictive calculations

Compound	Estimated log *P* (LogKow)	% absorbed dose in lymph	Reference
Testosterone	3.3	0.11	Ichihashi et al. (1994)
Progesterone	3.9	0.82	Ichihashi et al. (1994)
KEP	3.0	7.98	Sieber (1976)
KO	4.6	6.49	Ichihashi et al. (1994)
TE	6.2	12.48	Ichihashi et al. (1994)
DHTE	5.4	28.35	Ichihashi et al. (1994)
Δ^4-AE	6.6	96.77	Ichihashi et al. (1994)
Mepitiostaneolefin	5.1	97.67	Ichihashi et al. (1994)
Isotretinoin	6.8	1.99	Nankervis et al. (1996)
Etretinate	7.8	25.71	Nankervis et al. (1996)
Temarotene	8.7	45.67	Nankervis et al. (1996)
p,*p*-DDT	6.2	51.60	O'Driscoll et al. (1991)
Halofantrine	8.5	69.60	Caliph et al. (2000)
CI-976	5.8	43.00	Hauss et al. (1994)
Ontazolast	4.0	69.60	Hauss et al. (1998)
MK-386	8.0	1.67	Kwei et al. (1998)
Mepitiostane	6.0	92.59	Ichihashi et al. (1991)
Epitiostanol	4.4	4.53	Ichihashi et al. (1991)
Cyclosporine	3.0	2.16	Ueda et al. (1983)

found and reported, leading Ichihashi et al. (1994) to suggest that lipophilicity is an important factor in determining the extent to which drugs enter the lymph. However, further investigations of this have, to our knowledge, never been published.

Previous successes using in silico methods for predicting passive absorption (Palm et al., 1996; Krarup et al., 1998; Norinder et al., 1999), as well as the results reported by Ichihashi imply that in silico predictions may be applied on lymphatic transport. The aim of the present work is hence to develop a quantitative structure–lymphatic transfer relationship to predict the transfer of a lipophilic compound from the intestine to the lymph after dosing with a long-chain lipid vehicle.

The experimental values for distribution into the intestinal lymphatic system and the systemic circulation were taken from a number of literature references and are presented in Table 1. The data set covers a wide range of lymphatic transport as well as physicochemical properties. The inclusion criteria included: (i) the availability of data on the absorbed fraction lymphatically in rats, (ii) the availability of data on the absorbed fraction into the systemic circulation in rats, and (iii) co-administered with a long-chain triglyceride.

The molecular models were built with Sybyl molecular modelling system (Version 6.6) (Tripos Inc.) in extended conformations. Molecular descriptors were calculated with the VolSurf software (Version 2.0) (Cruciani et al., 2000a,b), where VolSurf calculates quantitative numerical descriptors extracted from the information present in these 3D molecular structures. The log *P* values were estimated using the on-line version of LogKow (Meyland and Howard, 1995).

The relationship between the experimental reported values for lymphatic transfer of xenobiotics in rats and the computed VolSurf descriptors were determined using partial least squares, projection to latent structures (PLS), which allows quantitative relations to be established between multiple variables (Wold et al., 1993). The software Simca-P (Version 8.0) was used for this purpose, transforming the lymphatic transfer data into log scale prior to the data analysis. Initially, all descriptors were included in the model and evaluated on basis of their variable influence on projection (VIP) values. Descriptors with low values were excluded and the effect on the model was judged by the cross-validated correlation coefficient (Q^2). If the value decreased, the descriptor in question was deselected. This improved the prediction of the lymphatic transfer.

Predicting the relationship between the physicochemical and the molecular properties when dosed in lipid-based delivery systems is important for the understanding of the absorption, distribution, metabolism and elimination processes. The animal

Table 2
Coefficient for predicting lymphatic transfer

	Coefficients
G	0.2635
ID3	0.2080
Emin3	−0.0004
W5	−0.0509
W6	−0.0761
HL1	−0.1396
Cw5	−0.1991
Iw8	−0.2266
Cw6	−0.2250
Constant	1.3057

models available involves microsurgery and stressful experiments so it is desirable to obtain a method, which in a simple way predicts the distribution into the intestinal lymphatic system while maintaining a satisfactory validity and consists of easily obtainable descriptors. On the basis of the VIP-values in Table 2 a two-component statistical significant model explaining the relationships between the molecular descriptors and the lymphatic transfer data were found. In the obtained model R^2X is 0.77; R^2Y is 0.83 and Q^2 is 0.73. R^2X and R^2Y are the variance of the experimental values, which is explained within the model (of the *X*s and the *Y*s) and Q^2 is a measure of the predictive power of the employed descriptors. The PLS analysis shows that lymphatic transfer data can be predicted based on nine descriptors and the formula coefficients are depicted in Fig. 1. When including the traditional predictor log *P* (represented by LogKow) in the multivariate data analysis the predictive power decreased and this value was thus not included in the model.

The descriptors used for the prediction are Cw5 and Cw6, which are the fraction of hydrophilic surface area; HL1, the hydrophilic–lipophilic ratio of the molecule; *G*, the globularity, which is 1.00 for a perfect sphere; W5 and W6, the size of the hydrophilic surface; Emin3, a local interaction minima; IW8, the unbalance between the centre of mass and the centre of the hydrophilic regions of the molecule; ID3, the unbalance between the centre of mass and the centre of the lipophilic regions of the molecule. A thorough review of the VolSurf descriptors can be found in the literature (Cruciani et al., 2000a,b).

Though many of the descriptors used in the model are highly correlated removing one of them will decrease the prediction power of the model. The number of descriptors seems high when compared to the number of compounds included, emphasizing that this model should be considered as an initial model. Furthermore, the model has not been validated using an external test set due to the limited data available.

The mean experimental errors in lymph and blood were obtained on 14 of the 19 compounds included in this study. The mean experimental error for the lymphatic transport was 29.8% (Fig. 2), and 22.8% for the absorption into the systemic circulation (Fig. 3). This error partly represents the small number of replicates performed in the in vivo experiments. Also the use of

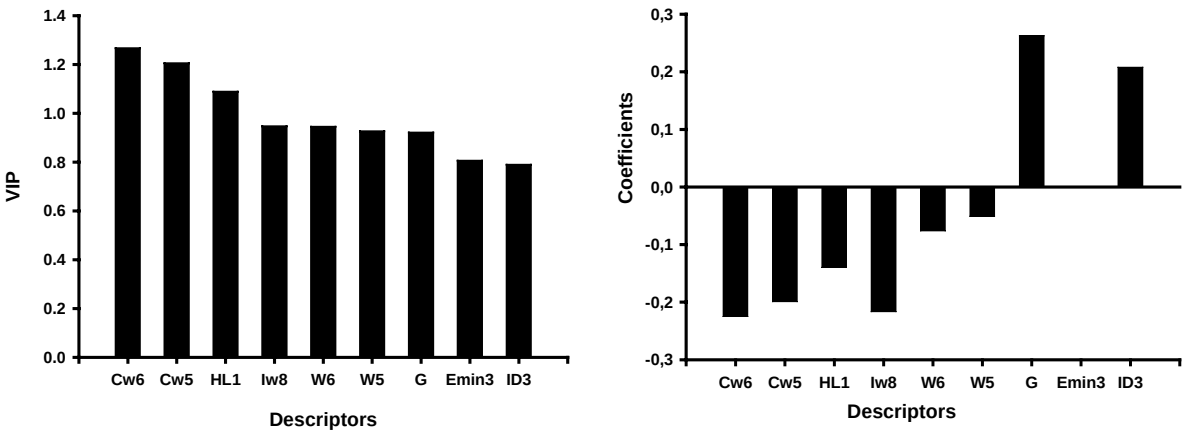

Fig. 1. Variable importance in projection and coefficients to illustrating the difference between the descriptors used to predict the lymphatic transfer.

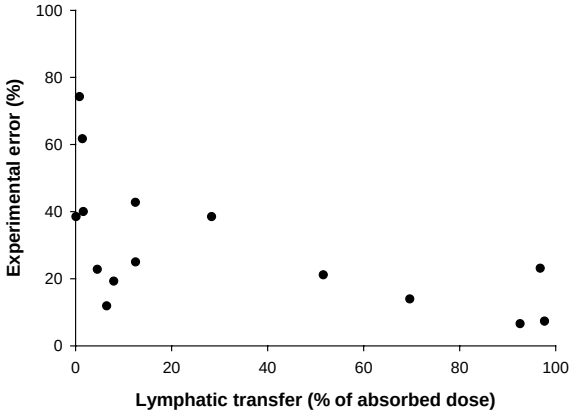

Fig. 2. Experimental error vs. experimental transfer into the intestinal lymphatic system in rats (% of absorbed dose) for 14 compounds.

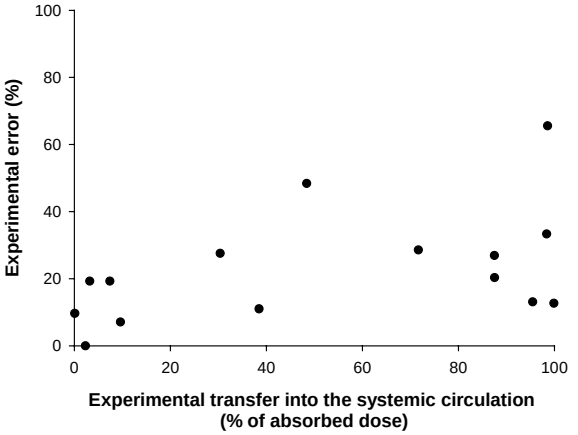

Fig. 3. Experimental error vs. experimental transfer into the systemic circulation in lymph cannulated rats (% of absorbed dose) for 14 compounds.

experimental data from a number of sources affects the predictive power of the final model, due to differences in the experimental protocols.

According to the developed model factors related to lipophilicity and size promoted high lymphatic transfer. This partly corresponds with the predictions reported by Charman and Stella (1986). These authors proposed that drug candidates for lymphatic transport should have a $\log P > 4.6$ and a triglyceride solubility >50 mg/ml. However, experimental results have shown that a combined high $\log P$ and a high triglyceride solubility does not always lead to significant lymphatic transport. Myers and Stella (1992) reported poor lymphatic transport of Penclomedine, an experimental cytotoxic agent with a $\log P$ of 5.48 and a triglyceride solubility of 175 mg/ml. Similarly, Hauss et al. (1994) reported low levels of lymphatic transport using CI-976, a lipophilic lipid regulator with a $\log P$ of 5.83 and a triglyceride solubility >100 mg/ml. Both these studies demonstrate the need for an altered predictive model for lymphatic transfer.

The computational model presented in the present paper can estimate the percentage of the absorbed dose to be transported lymphatically, when administered with a long-chain triglyceride at a higher predictive level than the $\log P$ values suggested by Ichihashi et al. (1994) (Fig. 4). This predictive model could

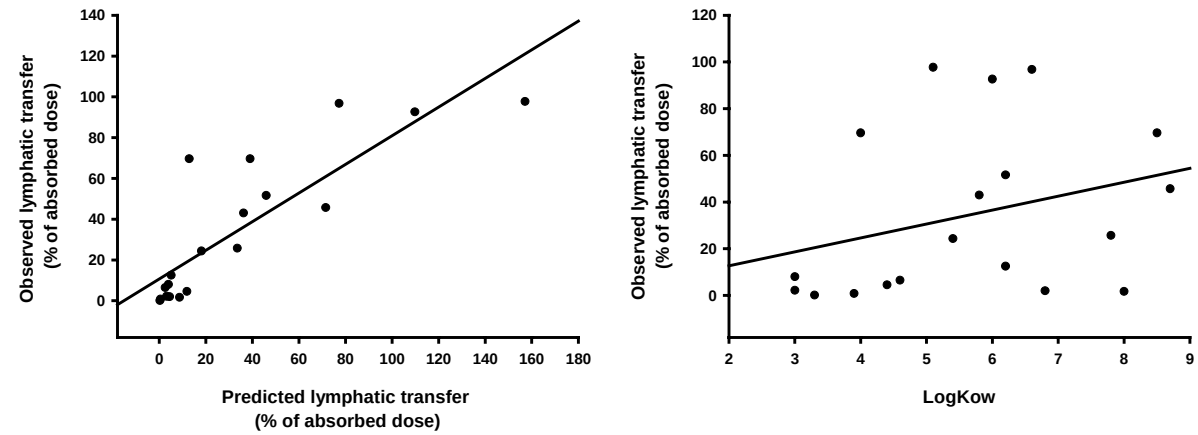

Fig. 4. Predicted vs. observed values and comparison to $\log P$ predictional power.

represent an easy accessible tool for evaluation of potential lymphatic transfer and hopefully initiate a renewed and expanded discussion of factors influencing the intestinal lymphatic transport—other than simple physicochemical parameters as log *P* and triglyceride solubility.

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