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Communications to the Editor

SPR Biosensor Studies of the Direct Interaction between 27 Drugs and a Liposome Surface: Correlation with Fraction Absorbed in Humans

Ernest Danelian,[†] Anders Karlén,[‡] Robert Karlsson,[†] Susanne Winiwarter,[‡] Anna Hansson,[†] Stefan Löfås,[†] Hans Lennernäs,[§] and Markku D. Hämäläinen*,[†]

Technical Operations, Biacore AB, Rapsgatan 7, SE-754 50 Uppsala, Sweden, and Departments of Organic Pharmaceutical Chemistry and Pharmacy, Uppsala Biomedical Centre, Uppsala University, SE-751 23 Uppsala, Sweden

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Introduction. Drugs are most commonly administered orally, and several biopharmaceutical, biochemical, and physiological factors will affect the drug fraction absorbed from the intestine (Fa). The biopharmaceutical factors of importance are stability, solubility (dissolution), and intestinal permeability. Since in vivo investigations of Fa in humans and animals are time-consuming and somewhat difficult to perform, there is a need for predictive in vitro models. For compounds being passively absorbed, Fa can be predicted using, for example, Caco-2 cells,[1] animal perfusion,[2] tissue diffusion,[3] liposome chromatography,[4] liposome partitioning,[5] and IAM (immobilized artificial membranes) chromatography[6] or with an assay based on microtiter filterplates covered with lecithin.[7] Recently, several studies have shown that Fa can be predicted from molecular properties calculated by computational methods.[8,9]

Most cell line approaches can only be used to predict passive transcellular diffusion. Active transport is only quantitatively predictable when carrier proteins are expressed in a sufficient amount in a cell line model. Experimental biological methods rely on the development of analytical procedures where the final detection is based on UV spectroscopy, mass spectrometry, or radiometry of labeled compounds. In addition, chromatographic techniques may have shortcomings, such as long retention times and/or peak broadening for lipophilic substances. The lack of a general detection method can also be a problem.

We describe here a new method to estimate the fraction of a drug absorbed in the human intestine based on biosensor technology. Liposomes are attached to a sensor surface, and the interactions between drugs and liposomes are monitored directly using surface plasmon resonance (SPR) technology. The phenomenon of SPR is sensitive to changes in refractive index at the sensor surface caused by changes in mass. The detection of an interaction does not therefore require chromophoric or radiolabeled compounds as do some other methods. Data obtained from this new method seem promising for use in the prediction of passive transcellular transport across the intestine, the most common drug absorption mechanism.

Results and Discussion. The 27 compounds used in this study (Table 1) were selected since reliable estimates of the human Fa values were available.[1,2,9−14] These compounds were classified according to their transport mechanism across the intestinal membrane (Table 1). Seventeen of the compounds were assumed to be absorbed transcellularly (t) (across the intestinal membrane), six of the compounds were absorbed by utilization of the paracellular (p) pathway (between the cells), and two were actively transported by carrier-mediated mechanisms (c). Two compounds were classified as using a combination of two transport routes. All compounds were also classified based on their degree of absorption (Table 1). Seventeen compounds with Fa > 70% were classified as highly absorbed (h), five compounds with Fa values between 30% and 70% as moderately absorbed (m), and five compounds with Fa values < 30% as having low absorption (l).[7] The 27 compounds had molecular weight values ranging from 60 to 504 Da.

BIACORE 3000 and Sensor Chip L1 were used to measure the interaction between liposomes and com-

* To whom correspondence should be addressed. Phone: 46-18-675761. Fax: 46-18-150113. E-mail: markku.hamalainen@biacore.com.
[†] Biacore AB.
[‡] Department of Organic Pharmaceutical Chemistry.
[§] Department of Pharmacy.

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Table 1. Drug Classification Data and Biosensor Results from POPC and POPC/GM1 Liposomes

drug	abbrev[a]	Fa (%)[b]	Fa_Cl[c]	transp[d]	POPC/GM1[e]	POPC[e]	MW[f]
desipramine	Desi_ht	100	h	t	1199	1009	266
verapamil	Vera_ht	100	h	t	1056	780	455
propranolol	Prop_ht	100	h	t	839	683	259
alprenolol	Alpr_ ht	96	h	t	480	297	249
sulfasalazine	Sulf_lt	12	l	t	348	241	398
pindolol	Pind_ht	92	h	t	314	142	248
oxprenolol	Oxpr_ht	97	h	t	277	120	265
naproxen	Napr_ht	100	h	t	183	109	230
metoprolol	Meto_ht	95	h	t	179	82	267
carbamazepine	Carb_ht	100	h	t	144	70	236
terbutaline	Terb_ht	73	h	t	117	47	225
ketoprofen	Keto_ht	100	h	t	101	62	254
hydrochlorothiazide	Hydr_mt	55	m	t	95	60	298
furosemide	Furo_mt	50	m	t	92	56	331
sulpiride	Sulp_mt	36	m	t	78	47	341
atenolol	Aten_mt	54	m	t	38	24	266
antipyrine	Anti_ht	100	h	t	12	19	188
tranexamic acid	Tran_mpt	55	m	pt	14	20	157
amoxicillin	Amox_hct	90	h	ct	9	17	365
foscarnet	Fosc_lp	17	l	p	62	32	126
raffinose	Raff_lp	0.3	l	p	34	24	504
lactulose	Lact_lp	0.6	l	p	29	22	342
mannitol	Mann_lp	26	l	p	29	22	182
urea	Urea_hp	100	h	p	26	23	60
creatinine	Crea_hp	100	h	p	13	22	113
L-leucine	Leuc_hc	100	h	c	11	18	131
D-glucose	Gluc_hc	100	h	c	10	18	180

[a] Abbrev: abbreviation combining name, Fa classification, and transport mechanism (see below). [b] Fa (%): fraction absorbed in humans (refs 1, 2, 9−14). [c] Fa_Cl: Fa classification: h = high, m = medium, l = low. [d] Transp: transport mechanism: t = passive transcellular, p = passive paracellular, c = carrier mediated. [e] POPC/GM1 and POPC: binding level for POPC/GM1 and POPC liposomes. [f] MW: molecular weight.

pounds. POPC (palmitoyl-oleoyl-phosphatidyl-choline) and POPC/GM1 (95/5) liposomes were attached to flow cells 4 and 3, respectively, and flow cells 1 and 2 were used as reference surfaces (Figure 1). Ganglioside GM1 was incorporated during liposome preparation to mimic a cell surface in which sugars are common components. During injection of a compound the SPR signal shows the binding of the compound to the liposomes, and at the end of injection, it shows the release of the compound from the liposome surfaces (Figure 2). Compounds were injected at a concentration of 500 μM.

The overlay plot in Figure 2, showing typical sensorgrams, demonstrates that steady-state levels are rapidly achieved and that there is a rapid release of compounds from the liposome surfaces. The response level after 80 s from start (nearly steady state) was used as a measure of binding in this study and is reported in Table 1. The strongly interacting drugs produced visually identifiable dissociation events.

Figure 3 shows the correlation between the signals from POPC/GM1 and POPC surfaces. The correlation is high but not equal to 1, which may indicate that additional information can be gained by using surface-modified liposomes. For example, differences in lipid headgroup charges may influence binding properties. The POPC surface has no net charge in contrast to the POPC/GM1 where the carboxylic group in GM1 creates a negative net charge to the lipid surface. In this particular case the graph indicates that POPC/GM1 has a slightly better resolution between l/m and m/h drugs. The discussion below is therefore based on the interaction between compounds and POPC/GM1 liposomes. Eleven out of the twelve transcellularly absorbed compounds with high intestinal absorption (Fa > 70%) gave signals ranging from 100 to 1200 RU. Antipyrine, which is classified as having high absorption, only gave a signal of 12 RU. The four transcellularly absorbed compounds classified as having medium absorption (Fa = 30−70%) gave signals in the range 40−100 RU.

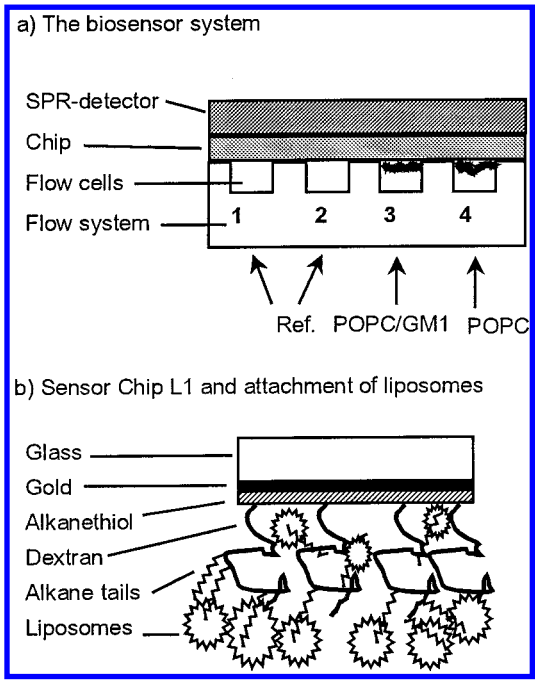

Figure 1. (a) Changes in SPR as a result of a change in mass/refractive index at the sensor surface indicate the binding of the drug candidate. In a BIACORE system individually and serially addressable flow cells allow simultaneous analysis from four positions on the surface. The drug is injected over the four flow cells, and the SPR system generates four sensorgrams. The signal from the reference surface is subtracted from that of the liposome surface to give a differential sensorgram (Figure 2). (b) Different liposomes are captured on alkane tails covalently attached to a dextran matrix.

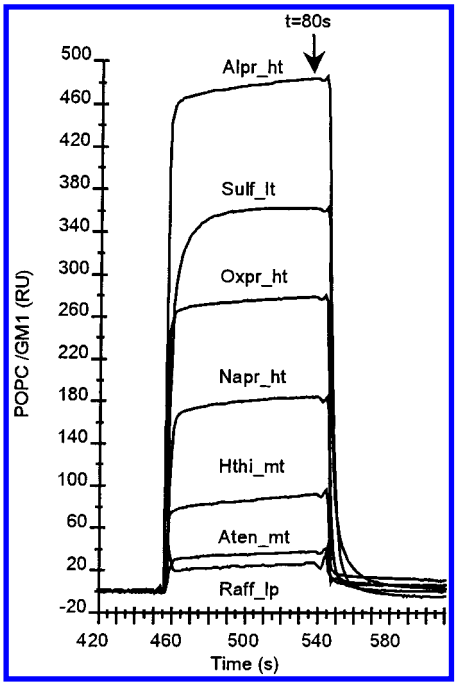

Figure 2. Seven drugs interacting with POPC/GM1 liposomes (start and end of injection at 455 and 545 s, respectively; for abbreviations, see Table 1).

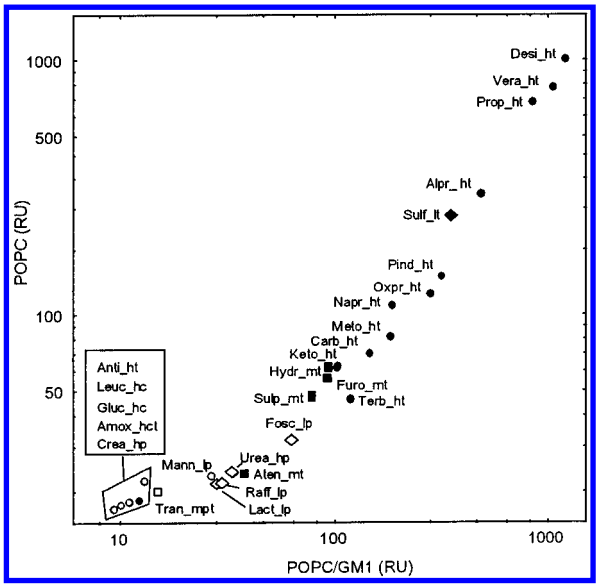

Figure 3. Scatter plot showing the correlation between the signals from POPC/GM1 and POPC liposomes (for abbreviations, see Table 1); filled symbols correspond to compounds classified as being absorbed via the transcellular route.

Finally, sulfasalazine, the only transcellularly absorbed compound classified as having low absorption (Fa < 30%), gave a high signal corresponding to 348 RU. Taken together, most of the compounds being passively absorbed via the transcellular route could be classified based on the POPC/GM1 signal in accordance with reported oral absorption data.

Figure 4 shows the correlation between the POPC/GM1 signal and fraction absorbed in humans (Fa). Filled symbols in this figure correspond to compounds being passively absorbed via the transcellular route. The moderately and highly absorbed compounds are clearly separated from each other, and a sigmoidal relationship to Fa can be seen. However, more compounds with small

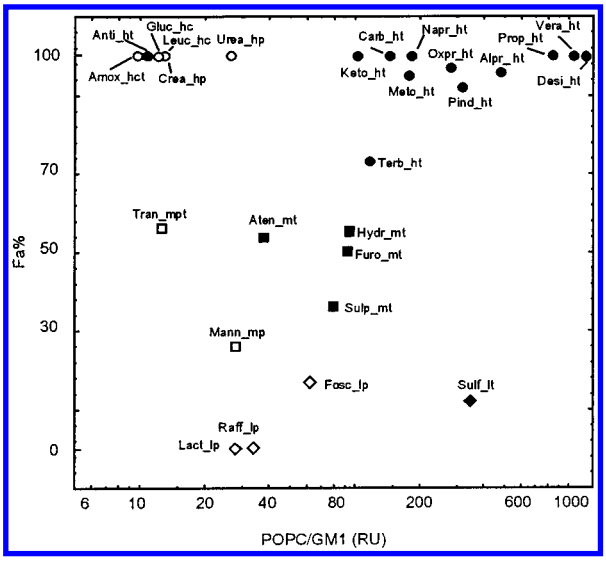

Figure 4. Correlation between the signal from POPC/GM1 and fraction absorbed in humans: circle, highly absorbed compounds with Fa > 70%; square, moderately absorbed compounds with Fa values between 30% and 70%; diamond, poorly absorbed compounds with Fa values < 30%; filled symbols correspond to compounds classified as being absorbed via the transcellular route.

Fa values need to be characterized using SPR technology in order to prove this relationship. The compounds in the upper-left corner of Figure 4 are all actively transported and/or use the paracellular route (except antipyrine). The outlier sulfasalazine is seen in the lower-right corner in the same figure.

Sulfasalazine and antipyrine were identified as outliers. Liposome binding data predict that sulfasalazine should be highly absorbed (Figure 2). However, after oral administration in humans the Fa value is low (12%),[9] and absorption is reported to be highly variable.[15] This difference may be due to the low stability of the compound in vivo and/or a significant efflux of sulfasalazine.[16] An alternative explanation for this compound is that the biosensor technology measures the binding event between the drug and the membrane but not an event related to transcellular transport itself. Antipyrine, the other outlier, has a high Fa value, but no interaction with liposome surfaces was observed. Antipyrine is commonly used as a dilution marker for body water in humans, which suggests a low binding to cell membranes in humans. The reasons for the high absorption in vivo are probably linked to the low molecular weight of the compound (188 Da) and the fact that it is not ionized under physiological conditions. Carbamazepine, also un-ionized under physiological conditions, gave a signal of 144 RU which indicates that this is not a general tendency for neutral compounds. Kansy et al.[7] reported that the filter plate assay had limited resolution for compounds below molecular weight 250 Da.

Conclusions. In the drug discovery process there is increased attention toward intestinal absorption properties and, in particular, to the discrimination between drugs with low and medium/high absorption. The increased number of hits from primary screens suggests that rapid methods are crucial to characterize compounds in secondary screening and lead optimization

phases. We have shown that a direct binding assay, using captured liposomes, provides data that predict Fa in humans for drugs using the transcellular absorption route. The majority of substances having Fa > 70% can be identified from liposome binding data. There are also indications that a group of drugs showing medium absorption can be identified. The drugs having MW < 200, where the paracellular diffusion route is possible, seem to be difficult to classify with this method. However, since the majority of drugs have a molecular weight above 200, this is considered to be a less important limitation.

The presented method can generate information from the interaction between a drug candidate and up to three different liposomes in one run. The liposome surfaces are very stable, and once prepared, a surface can be used for up to 2 weeks. The flexibility of the method is further increased by the fact that the liposome layer can be washed off with octylglycoside and the surface can then be restored by injecting new liposomes.

The throughput of the present method is 100 substances/24 h, testing a single concentration of each drug. Work is in progress to optimize the assay and increase the number of substances and molecular diversity. During the preparation of this manuscript we have seen indications that a concentration of 100−150 μM may be sufficient for correct classification of Fa properties. Using a lower concentration may also reduce potential solubility problems of the compounds.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Artursson, P.; Karlsson, J. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880−885.

(2) Fagerholm, U.; Johansson, M.; Lennernäs, H. Comparison between permeability coefficients in rat and human jejunum. *Pharm. Res.* **1996**, *13*, 1336−1342.

(3) Lennernäs, H.; Nylander, S.; Ungell, A.-L. Jejunal permeability: A comparison between the Ussing chamber technique and the single-pass perfusion in humans. *Pharm. Res.* **1997**, *14*, 667−671.

(4) Lundahl, P.; Beigi, F. Immobilized liposome chromatography of drugs for model analysis of drug-membrane interactions. *Adv. Drug Deliv. Rev.* **1997**, *23*, 221−227.

(5) Balon, K.; Riebesehl, B. U.; Müller, B. W. Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption. *Pharm. Res.* **1999**, *16*, 882−888.

(6) Pidgeon, C.; Ong, S.; Liu, H.; Qiu, X.; Pidgeon, M.; Dantzig, A. H.; Munroe, J.; Hornback, W. J.; Kasher, J. S.; Glunz, L.; Szczerba, T. IAM chromatography: An in vitro screen for predicting drug membrane permeability. *J. Med. Chem.* **1995**, *38*, 590−594.

(7) Kansy, M.; Senner, F.; Gubernator, K. Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes. *J. Med. Chem.* **1998**, *41*, 1007−1010.

(8) Winiwarter, S.; Bonham, N. M.; Ax, F.; Hallberg, A.; Lennernäs, H.; Karlén, A. Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. *J. Med. Chem.* **1998**, *41*, 4939−4949.

(9) Palm, K.; Stenberg, P.; Luthman, K.; Artursson, P. Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm. Res.* **1997**, *14*, 568−571.

(10) Lennernäs, H. Human jejunal effective permeability and its correlation with preclinical drug absorption models. *J. Pharm. Pharmacol.* **1997**, *49*, 627−638.

(11) Lennernäs, H. Human intestinal permeability. *J. Pharm. Sci.* **1998**, *87*, 403−410.

(12) *Clark's Isolation and Identification of Drugs*; Moffat, A. C., Ed.; The Pharmaceutical Press: London, 1986.

(13) *Therapeutic Drugs*; Dollery, C., Ed.; Churchill Livingstone: Edinburgh, 1991.

(14) The Fa value for creatinine was estimated and the values for propranolol and verapamil were adjusted based on the experimentally determined human jejunal P_{eff} values (ref 8) according to ref 2.

(15) Jack, D. B. *Handbook of Clinical Pharmacokinetic data*; Macmillan Publishers Ltd.: England, 1994.

(16) Liang, E.; Yazdanian, M. Investigation of sulfasalazine as a substrate of efflux pumps in caco-2 cells. *Pharm. Sci.* **1999**, *1*, 2564.

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