Note

Quantitative Structure–Activity Relationship Model for the Fetal–Maternal Blood Concentration Ratio of Chemicals in Humans

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A quantitative structure–activity relationship (QSAR) model of the fetal–maternal blood concentration ratio (F/M ratio) of chemicals was developed to predict the placental transfer in humans. Data on F/M ratio of 55 compounds found in the literature were separated into training (75%, 41 compounds) and testing sets (25%, 14 compounds). The training sets were then subjected to multiple linear regression analysis using the descriptors of molecular weight (MW), topological polar surface area (TopoPSA), and maximum E-state of hydrogen atom (Hmax). Multiple linear regression analysis and a cross-validation showed a relatively high adjusted coefficient of determination (R_a^2) (0.73) and cross-validated coefficient of determination (Q^2) (0.71), after removing three outliers. In the external validation, R^2 for external validation (R_{pred}^2) was calculated to be 0.51. These results suggested that the QSAR model developed in this study can be considered reliable in terms of its robustness and predictive performance. Since it is difficult to examine the F/M ratio in humans experimentally, this QSAR model for prediction of the placental transfer of chemicals in humans could be useful in risk assessment of chemicals in humans.

Key words placental transfer; human; quantitative structure–activity relationship; fetal–maternal blood concentration ratio

The placental transfer of chemicals is crucial for toxicity assessment of fetus, because chemicals that do not penetrate the placenta are considered safe for fetus.¹⁾ Therefore, investigations of many drugs that are transferred from mother to fetus through the placenta have been performed.

Since it is difficult to measure the placental transfer of chemicals in humans ethically, *in vitro* and *ex vivo* methods have been developed and used for the evaluation of human placental transfer.²⁾ In *in vitro* methods, cell lines from human placenta, such as BeWo, JAr, and JEG cells, have been commonly used as models of the placental barrier.³⁾ In *ex vivo* methods, the human placenta is perfused to investigate the transfer of chemicals from maternal to fetal compartment. Although this system has been validated in many studies, and therefore this system is considered reliable, there are difficulties in conducting this evaluation method.^{2,4)}

Quantitative structure–activity relationship (QSAR) models are useful tools for predicting the biological activity of chemicals. Descriptors of chemicals such as molecular weight and $\log P$, are needed to develop QSAR prediction, and those are determined by experiments, calculation, or predicted using software. In the field of pharmacokinetics, QSAR models have been developed and reported in areas such as oral bioavailability, Caco-2 permeability, and metabolism.^{5–7)}

QSAR models for predicting the placental transfer of chemicals using *ex vivo* data have already been reported.^{8,9)} However, to our knowledge, this is the first report that indicates prediction of the fetal–maternal blood concentration ratio (F/M ratio) of chemicals using *in vivo* data. To predict the F/M ratio of chemicals in humans, we developed a QSAR model using the data of umbilical cord/maternal blood concentration ratio, derived from information found in the literature. Our

model could be useful in fetal toxicity assessment.

MATERIALS AND METHODS

Data Sets The F/M ratio of 55 compounds was used as data sets.^{10–25)} Although the F/M ratio of many compounds was directly cited from literature sources such as clinical studies, the ratio for some compounds (carnitine, chlordane, chlorpyrifos, dichlorodiphenyldichloroethylene (DDE), diazinon, dicloran, hexachlorobenzene (HCB) heptachlor epoxide, nonachlor, oxychlordane, and phthalimide) was calculated based on the concentration in umbilical cord and maternal blood.

Descriptors PaDEL-descriptor (version 2.18) is a free software application developed at the National University of Singapore, which can calculate 770 2D descriptors.²⁶⁾ Regarding the membrane penetration, the importance of molecular size and lipophilicity of compounds has been reported, and thus for the analysis, the descriptors of molecular weight (MW) and $X \log P$ were selected.^{8,9)} In addition, the descriptors of sum of the atomic polarizabilities (Apol), autocorrelation of a topological structure (ATS) descriptor, weighted by charges (ATSc1, ATSc2, ATSc3, ATSc4, ATSc5), ATS descriptor, weighted by polarizability (ATSp1, ATSp2, ATSp3, ATSp4, ATSp5), sum of the absolute value of the difference between atomic polarizabilities of all bonded atoms in the molecule (Bpol), maximum E-state of hydrogen atom (Hmax), maximum E-state (Gmax), minimum E-state of hydrogen atom (Hmin), minimum E-state (Gmin), Global topological charge index (GlobalTopoChargeIndex), combined dipolarity/polarizability (MLFER_S), and topological polar surface area (TopoPSA) were chosen because those parameters are in-

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volved in the polarity of chemicals.^{27–31)} Descriptors for which correlation coefficients for other descriptors of the training sets were more than 0.7 were eliminated to avoid the multicollinearity. Finally, the descriptors of ATSc3, ATSc4, ATSp1, Hmax, Gmax, Hmin, GlobalTopoChargeIndex, TopoPSA, MW, and $X \log P$ were used to develop the QSAR model.

Development of *in Silico* Prediction Model The data sets were separated into training (75%) and testing sets (25%) using random number generation. Training sets were subjected to multiple linear regression analysis, using R programming language (version 3.1.0). The model with the lowest Akaike Information Criterion (AIC) was generated by a backward selection procedure using 10 descriptors.³²⁾ Subsequently, *p* values (0.05) of significance tests for the regression coefficients were referenced to select descriptors. As the cross-validation of the model, Leave-one-out Cross-Validation (LOO-CV) was

Fig. 1. A Plot of Observed vs. Predicted log(F/M) Ratio of Training Sets

Solid triangles indicate outliers.

Table 1. Names, Descriptors, and Observed and Predicted log(F/M) Ratio of Training Sets

Name	Hmax	TopoPSA	MW	Observed log(F/M)	Predicted log(F/M)	Reference
17-Hydroxyprogesterone caproate	0.582	60.4	428	-0.70	-0.63	10
Abacavir	0.689	101.9	286	0.01	-0.16	11
Acebutolol	0.753	87.7	336	-0.10	-0.29	12
Buprenorphine	1.250	62.2	467	-0.46	-0.46	15
Carnitine	0.670	60.4	161	0.11	0.02	16
Cefoperazone	1.015	264.3	648	-0.46	-0.49	17
Chlordane	0.230	0.0	406	-0.78	-0.85	18
Chloroquine	0.544	28.2	319	-0.03	-0.47	17
Chlorpyrifos	1.154	82.5	349	-0.01	-0.18	19
Clindamycin	0.814	127.6	424	-0.30	-0.37	17
Clonazepam	0.841	84.6	315	-0.23	-0.21	17
DDE	0.407	0.0	316	-0.98	-0.58	18
Diazepam	0.791	32.7	284	0.10	-0.28	14
Dicloran	0.674	69.2	206	0.03	-0.06	19
Didanosine	0.849	88.7	236	-0.42	-0.02	11
Ethabutol	0.577	64.5	204	-0.12	-0.10	17
Etidocaine	0.637	32.3	276	-0.47	-0.32	21
Flecainide	1.391	59.6	414	-0.20	-0.29	17
Flupenthixol	1.339	52.0	434	-0.62	-0.37	17
HCB	0.150	0.0	282	-0.70	-0.60	18
Heptachlor epoxide	0.468	12.5	386	-0.48	-0.69	18
Indinavir	0.795	118.0	613	-1.10	-0.84	14
Isoniazid	0.675	68.0	137	-0.21	0.10	17
Lamivudine	0.819	113.5	229	-0.03	0.05	11
Lidocaine	0.637	32.3	234	-0.26	-0.23	17
Metronidazole	0.735	81.2	171	0.00	0.07	17
Mifepristone	0.595	40.5	429	-0.96	-0.67	17
Naloxone	0.890	70.0	327	-0.30	-0.26	17
Nelfinavir	0.768	127.2	567	-0.60	-0.72	23
Nevirapine	0.624	58.1	266	-0.17	-0.24	22
Nifedipine	1.039	107.8	346	-0.11	-0.15	24
Nonachlor	0.228	0.0	440	-0.84	-0.93	18
Phthalimide	0.635	46.2	147	-0.09	0.01	19
Piperacillin	1.052	181.7	517	-0.57	-0.37	17
Propranolol	0.707	41.5	259	-0.59	-0.23	14
Remifentanyl	0.701	76.2	376	-0.14	-0.43	25
Sotalol	1.019	86.8	272	0.00	-0.04	12
Stavudine	0.931	78.9	224	0.12	0.02	11
Ticarcillin	0.980	177.6	384	-0.04	-0.10	17
Valproic acid	0.669	37.3	144	0.18	0.01	14
Zidovudine	0.945	91.2	267	0.09	-0.05	11

conducted to evaluate the predictive performance, and the cross-validated coefficient of determination (Q^2) was used as the indicator.³³⁾ In addition, an external validation was made, which is considered the best method of evaluation of the model, and R^2 for external validation (R^2_{pred}) was used as the indicator.³³⁾

RESULTS AND DISCUSSION

Multiple Linear Regression Analysis By the AIC backward selection procedure and the selection based on p values (0.05) of significance tests for the regression coefficients, 3 descriptors (MW, TopoPSA, and Hmax) were selected to develop the model of training sets (41 compounds). MW and TopoPSA are the descriptors of molecular size and polar surface area of compounds, respectively, and involved in the membrane penetration of placenta.^{8,9)} Votano *et al.* reported that Hmax is the maximum hydrogen atom E-state value in a compound, and correlates with charge.³⁴⁾ For example, the hydrogen atoms of OH, NH₂, and COOH groups tend to be Hmax. However, the involvement of Hmax for the placental transfer has not been reported.

Multiple linear regression analysis of training sets and a cross-validation were conducted as described below (Eq. 1) (Fig. 1, Table 1). Values in parentheses indicate the 95% confidence limits.

$$\begin{aligned} \log(F/M) = & -0.00231(\pm 0.000581)\text{MW} \\ & + 0.00233(\pm 0.00160)\text{TopoPSA} \\ & + 0.382(\pm 0.285)\text{Hmax} - 0.00363 \\ n = 41, R_a^2 = 0.64, Q^2 = 0.63 \end{aligned} \quad (1)$$

Because the absolute values of the standardized residuals of three compounds (chloroquine, DDE, and didanosine) were more than two, these compounds were considered outliers. After removing these three outliers, multiple linear regression analysis and a cross-validation were conducted again (Eq. 2). Values in parentheses indicate the 95% confidence limits.

$$\begin{aligned} \log(F/M) = & -0.00238(\pm 0.00050)\text{MW} \\ & + 0.00238(\pm 0.00139)\text{TopoPSA} \\ & + 0.380(\pm 0.245)\text{Hmax} + 0.0283 \\ n = 38, R_a^2 = 0.73, Q^2 = 0.71 \end{aligned} \quad (2)$$

To clarify the importance of the descriptors used in multiple linear regression analysis, the standard partial regression coefficients were calculated, and those values for MW, TopoPSA, and Hmax were -0.922 , 0.376 , and 0.314 , respectively. These results suggest that the MW descriptor is more important for the F/M ratio in humans than the polarity descriptors.

The MW descriptor was negative-valued in our prediction model, in common with an *ex vivo* model.⁹⁾ This means that for decreasing values of MW, the F/M ratio increases. Since small compounds penetrate the placental membrane more easily than large compounds, the negative value of MW seems reasonable in terms of the F/M ratio.⁹⁾ On the other hand, the polarity descriptors (TopoPSA and Hmax) were positive-valued in the model, suggesting that when TopoPSA and Hmax decrease the F/M ratio also decreases. It should be noted that in the *ex vivo* model for prediction of the F/M ratio, those descriptors were negative-valued, different from our model. In the *ex vivo* model, the F/M ratio is decided based only on the effect of membrane penetration, because a buffer is used

Fig. 2. The Plot of Observed vs. Predicted log(F/M) Ratio of Testing Sets

Solid triangles indicate outliers.

Table 2. Names, Descriptors, and Observed and Predicted log(F/M) Ratio of Testing Sets

Name	Hmax	TopoPSA	MW	Observed log(F/M)	Predicted log(F/M)	Reference
Atazanavir	0.910	171.2	704	-0.89	-0.90	13
Bupivacaine	0.637	32.3	288	-0.52	-0.34	14
Clonidine	0.492	36.4	229	-0.05	-0.24	17
Diazinon	1.173	95.4	304	0.00	-0.02	19
Disopyramide	0.662	59.2	339	-0.59	-0.39	17
Duloxetine	0.701	49.5	297	-0.92	-0.29	20
Indomethacin	0.820	68.5	357	-0.01	-0.35	17
Lopinavir	0.902	120.0	628	-0.62	-0.84	22
Metoprolol	0.691	50.7	267	0.00	-0.22	12
Midazolam	0.785	29.7	325	-0.13	-0.38	17
Norbuprenorphine	0.768	71.0	413	-0.31	-0.49	15
Oxprenolol	0.727	50.7	265	-0.43	-0.21	12
Oxychlorane	0.465	12.5	420	-1.02	-0.76	18
Procainamide	0.549	58.4	235	0.04	-0.18	17

in the *ex vivo* experimental system instead of umbilical cord and maternal blood, and the impact on the interaction of compounds with blood lipids is ignored. Thus, under the *ex vivo* conditions, compounds of low polarity would be more easily transported across the placental membrane than those of high polarity, because of the high lipid content in the membrane, leading to the negative values of the polarity descriptors.

In contrast, the determination of the *in vivo* F/M ratio was based on the influence of both membrane penetration and interaction with blood lipids. Because maternal blood contains several times higher lipid content than newborn blood, compounds of low polarity would more easily exist in the maternal blood.³⁵⁾ In addition, uptake transporters may be involved in the placental transfer of chemicals.³⁶⁾ For example, it was reported that the placental transfer of valproic acid is mediated by monocarboxylic acid transporters (MCT).³⁷⁾ In general, substrates of membrane transporters are considered hydrophilic.³⁸⁾ Therefore, the positive values of the polarity descriptors (TopoPSA and Hmax) might be attributed to uptake transporters. These findings suggest that the F/M ratio of chemicals *in vivo* would be described on the basis of both membrane penetration and interaction with blood lipids.

External Validation Because the data sets were separated into training (75%) and testing sets (25%) using random number generation, external validation was conducted using testing sets (14 compounds), showing that R^2_{pred} was 0.51, which is considered an acceptable model³³⁾ (Fig. 2, Table 2).

CONCLUSION

A QSAR model for prediction of the placental transfer in humans was developed using F/M ratio of 55 compounds. The model for training sets (41 compounds) was developed using multiple linear regression analysis with three descriptors (MW, TopoPSA, and Hmax). The predictive ability of the model was confirmed by external validation (14 compounds). This QSAR model could be useful in risk assessment of placental transfer of chemicals to fetus, because it is difficult to determine the F/M ratio of humans experimentally.

Conflict of Interest Tomoyuki Takaku, Hirohisa Naga-hori, and Yoshihisa Sogame are employees of Sumitomo Chemical Co., Ltd.

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