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Molecular Determinants Responsible for Sedative and Non-sedative Properties of Histamine H_1–Receptor Antagonists

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Abstract. There is argument whether non-sedative properties of histamine H_1–receptor antagonists (antihistamines) are determined by their active extrusions from the brain via P-glycoprotein or their restricted penetration through the blood-brain barrier. We have reported that sedative and non-sedative antihistamines can be well discriminated by measuring changes in their binding to H_1 receptors upon receptor internalization in intact cells, which depends on their membrane-penetrating ability. In this study, molecular determinants responsible for sedative and non-sedative properties of antihistamines were evaluated by quantitative structure-activity relationship (QSAR) analyses. Multiple regression analyses were applied to construct a QSAR model, taking internalization-mediated changes in the binding of antihistamines as objective variables and their structural descriptors as explanatory variables. The multiple regression model was successfully constructed with two explanatory variables, i.e., lipophilicity of the compounds at physiological pH (logD) and mean information content on the distance degree equality (IDDE) ($r^2 = 0.753$). The constructed model discriminated between sedative and non-sedative antihistamines with 94% accuracy for external validation. These results suggest that logD and IDDE concerning lipophilicity and molecular shapes of compounds, respectively, predominantly determine the membrane-penetrating ability of antihistamines for their side effects on the central nervous system.

Keywords: histamine H_1 receptor, antihistamine, sedation, membrane penetration, P-glycoprotein

Introduction

Histamine H_1–receptor antagonists / inverse agonists (antihistamines) are well known to have side effects such as sedation, hypnosis, and cognitive impairment, which are associated with the blockade of H_1 receptors in the central nervous system (CNS). On the basis of these clinical side effects, antihistamines are generally divided into two groups, i.e., sedative and non-sedative (or less sedative) antihistamines. Non-sedative antihistamines have fewer side effects on the CNS as a result of less blockade of H_1 receptors in the CNS, although they might induce sedation at higher doses. It is yet still inconclusive, however, about whether non-sedative properties of antihistamines are determined by their active extrusion from the brain via P-glycoprotein (1 – 3) or their restricted penetration through the blood–brain barrier (4 – 7).

Receptor internalization, movement of the receptor from the cell surface to intracellular compartments, is known to affect the binding properties of receptor ligands in intact cells, depending on their ability to penetrate the biomembrane (8, 9). We therefore tested how receptor internalization influenced the binding properties of a variety of antihistamines under ice-cold conditions where a P-glycoprotein–mediated extrusion pump might not work (10). Our finding is that there are clear differences between the effect of H_1-receptor internalization on the binding of sedative and non-sedative antihistamines to intact cells, which provide strong evidence that simple diffusion through the plasma membrane predominantly determines their sedative and non-sedative properties. However, the variety of chemical structures and physicochemical properties of antihistamines makes it difficult

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to accurately predict membrane-penetrating ability for their sedative and non-sedative properties.

Quantitative analyses of the chemical structures of compounds can be useful to explain and predict their effects on physiological functions (11): many studies on quantitative structure–activity relationships (QSAR) have been successful in the analyses and prediction of pharmacological effects (12), enzymatic activities (13), affinities for receptor proteins (14), pharmacokinetic parameters (15), and drug metabolism (16). However, there is no report concerning a QSAR model specifically designated for the membrane-penetrating ability of antihistamines; therefore, we tried to establish a QSAR model to explain and predict membrane-penetrating ability of antihistamines for their sedative and non-sedative properties on the basis of our previous report (10).

Here we show that sedative and non-sedative properties of antihistamines can be predicted with extremely high accuracy by the QSAR model constructed on the basis of their membrane-penetrating ability alone. To our knowledge, we succeeded for the first time in constructing a QSAR model to explain and predict sedative and non-sedative properties of antihistamines, and the constructed QSAR model may also contribute to optimizing the development of novel antihistamines with respect to their side effects on the central nervous system.

Materials and Methods

Training and external validation set of antihistamines assessed

Nineteen antihistamines, for which the internalization-mediated changes in their binding to intact cells are already known (10), were assessed as a training set of antihistamines (Fig. 1): sedative antihistamines were chlorpheniramine, clemastine, cyproheptadine, diphenhydramine, mepyramine, promethazine, azelastine, ketotifen oxatomide, ebastine, loratadine, and terfenadine (12 compounds) and non-sedative antihistamines were mequitazine, epinastine, bepotastine, carebastine, fexof-

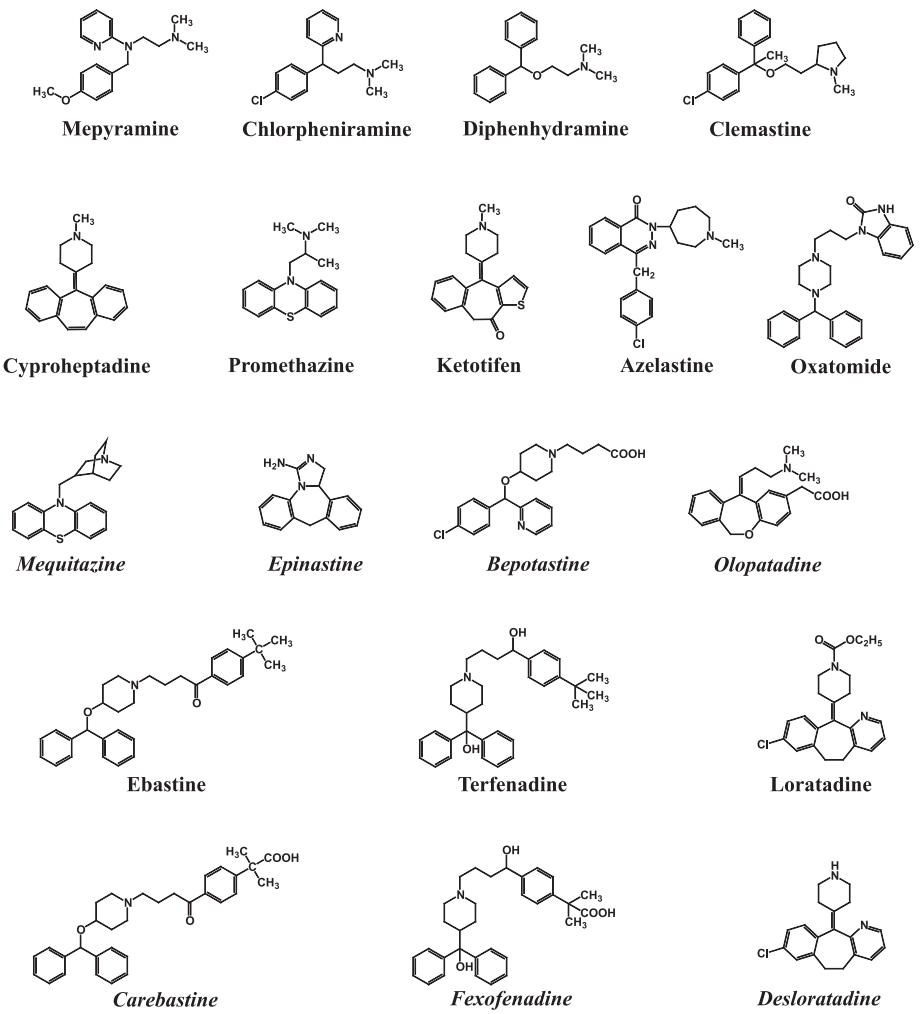

Fig. 1. Chemical structures of antihistamines assessed as a training set. Chemical structures of 19 antihistamines are shown. The training set of antihistamines consisted of 12 sedative and 7 non-sedative (in italics) compounds.

enadine, desloratadine, and olopatadine (7 compounds). The non-sedative behavior of ebastine, loratadine, and terfenadine is considered to be due to their corresponding active metabolites, carebastine, desloratadine, and fexofenadine, respectively (10).

Sixteen antihistamines, for which the internalization-mediated changes in their binding to intact cells are unknown, were assessed as an external validation set of antihistamines (Fig. 2): sedative antihistamines were alimemazine, azatadine, dimetindene, diphenylpyraline, homochlorcyclizine, hydroxyzine, imipramine, isothipendyl, and triprolidine (9 compounds) and non-sedative antihistamines were acrivastine, astemizole, cetirizine, emedastine, levocabastine, mizolastine, and temelastine (7 compounds).

Objective variables for assessment of sedative and non-sedative properties of antihistamines

As an objective variable to assess the sedative and non-sedative properties of antihistamines, the extent of changes in the binding of a training set of 19 antihistamines by internalization of H_1 receptors was expressed as the difference in area under the curve (AUC) between the displacement curves obtained with histamine-pretreated (i.e., internalization-induced) and histamine–non-pretreated control cells (*Δ*AUC, Fig. 3).

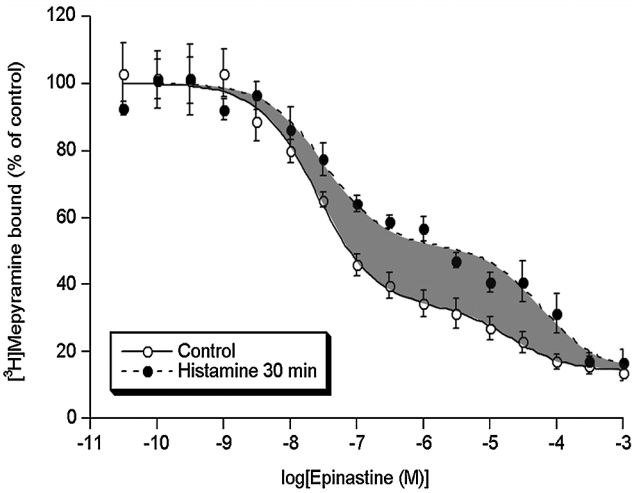

Fig. 3. *Δ*AUC as an objective parameter to quantitatively assess internalization-mediated changes in displacement curves for antihistamines against [^{3}H]mepyramine binding to H_1 receptors. The figure shows displacement curves for a non-sedative H_1-receptor antagonist, epinastine, against [^{3}H]mepyramine binding to intact U373 MG astrocytoma cells (taken from our previous paper, ref. 10, with permission). *Δ*AUC was expressed as the difference in AUC between the displacement curves obtained with histamine-pretreated [i.e., internalization-induced (closed circle) and histamine–non-pretreated control cells (open circle)].

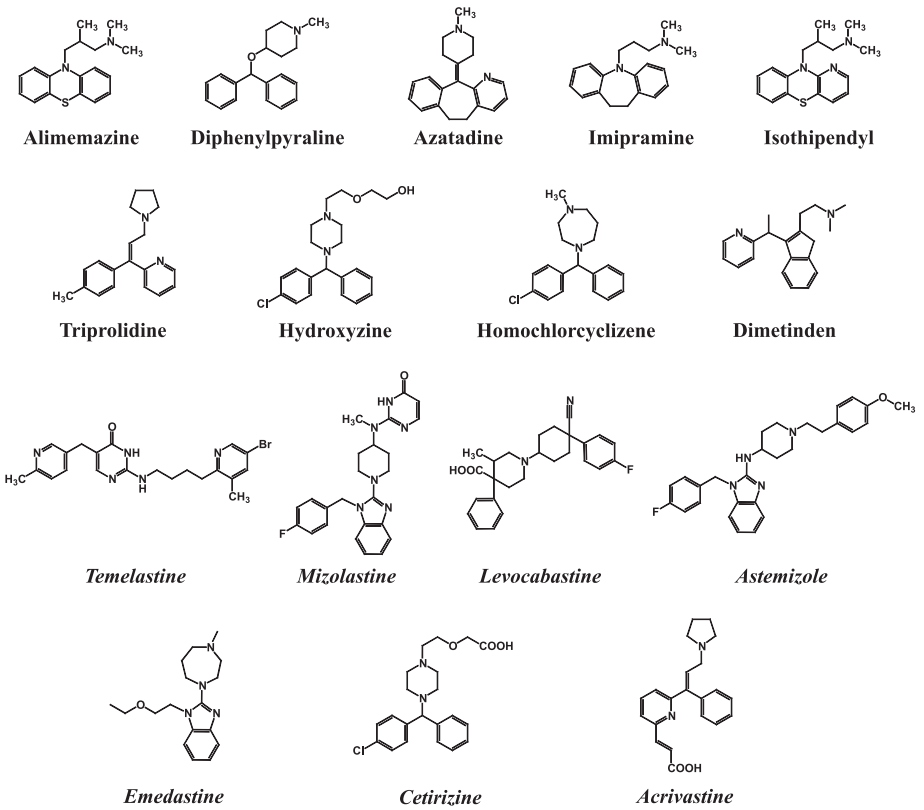

Fig. 2. Chemical structures of antihistamines assessed as an external validation set. Chemical structures of 16 antihistamines are shown. The external validation set of antihistamines consisted of 9 sedative and 7 non-sedative (in italics) compounds.

Briefly, cells were pretreated with or without 0.1 mM histamine for 30 min at 37°C in HEPES buffer (120 mM NaCl, 5.4 mM KCl, 1.6 mM $MgCl_2$, 1.8 mM $CaCl_2$, 11 mM D-glucose, and 25 mM HEPES, pH 7.4 at 37°C) to induce the internalization of H_1 receptors. Subsequently, the cells were washed with ice-cold HEPES buffer and intact cell binding was performed at 4°C as described in our previous paper (9, 10). The displacement curves were fitted to either a one- or two-site model as follows (KaleidaGraph; Synergy Software, Reading, PA, USA);

One-site model in the experiment without histamine pretreatment: $B1_{cont} = 100 - (P \times C) / (IC_{cont} + C)$

One-site model in the experiment with histamine pretreatment: $B1_{his} = 100 - (P \times C) / (IC_{his} + C)$

Two-site model in the experiment without histamine pretreatment: $B2_{cont} = 100 - (P_H \times C) / (IC_{contH} + C) - (P_L \times C) / (IC_{contL} + C)$

Two-site model in the experiment with histamine pretreatment: $B2_{his} = 100 - (P_H \times C) / (IC_{hisH} + C) - (P_L \times C) / (IC_{hisL} + C)$

, where $B1_{cont}$, $B1_{his}$, $B2_{cont}$, and $B2_{his}$ are the amounts of bound [^{3}H]mepyramine (taking radioactivity in the absence of antihistamines as 100% in each set of experiments); P is the percentage of the binding of antihistamines; C is the concentration of antihistamines used; IC_{his} and IC_{cont} are the IC_{50} values for antihistamines in cells with or without histamine pretreatment, respectively; P_H and P_L are percentages of high and low affinity sites for antihistamines, respectively; IC_{contH} and IC_{contL} are IC_{cont} values for antihistamines at high and low affinity sites, respectively; IC_{hisH} and IC_{hisL} are IC_{his} values for antihistamines at high and low affinity sites, respectively. ΔAUC for each antihistamine in the training set was calculated as follows:

$\Delta AUC1 = \int B1_{his}dx - \int B1_{cont}dx$

$\Delta AUC2 = \int B2_{his}dx - \int B2_{cont}dx$

, where ΔAUC1 was defined as ΔAUC introduced from $B1_{cont}$ and $B1_{his}$ in the one-site model; ΔAUC2 was defined as ΔAUC introduced from $B2_{cont}$ and $B2_{his}$ in the two-site model.

Descriptors as explanatory variables for ΔAUC

Chemical structures of antihistamines were collected in the "SMILES" format from the NCBI PubChem compound database. Three-dimensional structures were constructed by "clean 3D function, process for energy optimization of 3D structures" in Marvin View ver. 5.3.2 (ChemAxon, Ltd., Budapest, Hungary) from the SMILES files. Geometries of the 3D structures were refined and optimized by MMFFaq force field in Spartan 08 ver. 1.1.1. (Wavefunction, Inc., Irvine, CA, USA). Molecular descriptors of antihistamines were calculated from their optimized 3D structures by Dragon software ver. 5.5 (Talete srl, Milano, Italy). The Dragon descriptors of 1593 types were used in the present study. Lipophilicity of the compounds at pH 7.5 (logD) was calculated by a tautomer-considered KLOP method in arvinView.

Construction and application of simple and multiple regression models

The Dragon descriptors and logD values were used to construct simple and multiple regression models in the training set. The best model was explored by genetic algorithms (17) with the leave-one-out cross validation (18) as a selection pressure of the model using MobyDigs ver 1.1 (Talete srl); that is, the determination coefficient in the leave-one-out method (Q^2loo) was used as an index of predictive performance in the model. The predictive performance in the model was validated by the prediction of bootstrapping samples of compounds in the training set. Finally, the constructed model was applied to predict sedative and non-sedative antihistamines in the external validation set.

Regression diagnosis

Regression diagnosis and other statistical analyses of the prediction models were performed by JMP ver. 8.0.2 (SAS Institute, Inc., Cary, NC, USA).

Results

ΔAUC as an objective variable for assessment of sedative and non-sedative properties of antihistamines

In the training set, sedative and non-sedative antihistamines were successfully discriminated using experimentally-obtained ΔAUC values with a value of approximately 20 arbitrary units (Table 1 and Fig. 4).

Simple regression analyses

Simple regression analyses (SRA) showed that logD had the most significant correlation with ΔAUC values for antihistamines in the training set and ΔAUC values predicted by logD were as follows (Table 1):

SRA-predicted ΔAUC =

$-(9.87 \pm 2.02)\text{logD} + (44.2 \pm 6.1)$

A significant relationship between ΔAUC values obtained experimentally and predicted by the above equation was also confirmed (Fig. 5a; $n = 19$, $r^2 = 0.584$).

Using this equation, sedative and non-sedative antihistamines in the external validation set were discriminated with 75% accuracy at the value of 20 arbitrary units of SRA-predicted ΔAUC (Fig. 6a; 12 of 16 antihistamines).

Table 1. Obtained parameters for antihistamines

				⊿AUC		
	Antihistamines	logD	IDDE	Experimentally-obtained	SRA-predicted	MRA-predicted
Training set	Azelastine	2.64	4.31	14.76	18.13	26.28
	Chlorpheniramine	1.65	3.43	17.36	27.93	15.95
	Clemastine	3.42	4.25	11.77	10.41	17.18
	Cyproheptadine	3.19	3.64	1.82	12.75	5.48
	Diphenhydramine	2.49	2.95	0.88	19.66	−3.57
	Ketotifen	2.87	3.75	−8.53	15.90	11.16
	Mepyramine	1.76	3.52	8.15	26.80	16.89
	Oxatomide	3.36	3.83	−0.70	11.05	8.08
	Promethazine	2.92	3.52	5.23	15.40	5.42
	Ebastine	6.21	4.19	−2.11	−17.09	−11.82
	Loratadine	4.13	4.61	10.46	3.41	18.31
	Terfenadine	5.27	4.19	10.53	−7.80	−2.49
	Bepotastine	0.16	4.13	34.88	42.59	46.82
	Carebastine	1.99	4.27	28.85	24.60	31.86
	Desloratadine	1.59	4.15	39.02	28.53	33.09
	Epinastine	0.58	4.14	75.43	38.48	42.89
	Fexofenadine	1.06	4.27	35.26	33.69	40.99
	Mequitazine	3.14	3.74	23.23	13.20	8.24
	Olopatadine	−1.93	4.24	74.61	63.26	70.13
External validation set	Alimemazine	2.64	3.33	—	18.19	3.85
	Azatadine	2.87	3.64	—	15.87	8.62
	Dimetindene	1.20	4.01	—	32.40	33.61
	Diphenylpyraline	2.40	3.25	—	20.47	4.24
	Homochlorcyclizine	2.82	4.10	—	16.40	19.62
	Hydroxyzine	3.14	4.16	—	13.21	17.92
	Imipramine	2.83	3.63	—	16.32	8.82
	Isothipendyl	2.39	3.52	—	20.65	10.69
	Triprolidine	2.69	3.73	—	17.71	12.42
	Acrivastine	−0.57	4.16	—	49.79	54.70
	Astemizole	2.00	4.62	—	24.45	39.68
	Cetirizine	0.27	4.13	—	41.54	45.76
	Emedastine	1.31	4.46	—	31.28	42.92
	Levocabastine	2.19	4.44	—	22.57	33.68
	Mizolastine	2.95	4.56	—	15.10	29.05
	Temelastine	2.38	4.25	—	20.70	27.55

Values of ⊿AUC, logD, and IDDE for antihistamines were obtained as described in Materials and Methods. AUC, area under the curve; logD, lipophilicity of the compounds at pH 7.5; IDDE, the mean information content on distance degree equality; SRA, simple regression analyses; MRA, multiple regression analyses.

Multiple regression analyses

In multiple regression analyses (MRA), combinatorial optimization of the descriptors was performed to explain the variance of ⊿AUC by a genetic algorithm with an adequate number of trials. As a result, a regression formula with the maximal determination coefficient in the leave-one-out cross-validation (Q^2loo) was constructed as below, and a more significant relationship was observed between ⊿AUC values obtained experimentally and predicted in MRA than in SRA (n = 19, $r^2 = 0.742$, $Q^2loo = 63.8$, $Q^2boot = 65.5$, $P < 0.0001$, $F = 23.0$, $s = 12.7$) (Table 1 and Fig. 5b):

MRA-predicted ⊿AUC = −(9.90 ± 1.64)logD + (23.0 ± 7.3)IDDE − (46.6 ± 29.4)

, where IDDE indicates the mean information content on distance degree equality. Bootstrap validation also confirmed the above equation with the maximal determination coefficient (Q^2boot). Standardized partial regression coefficients of logD and IDDE in the regression equation were −0.768 and 0.398, respectively. On the other hand,

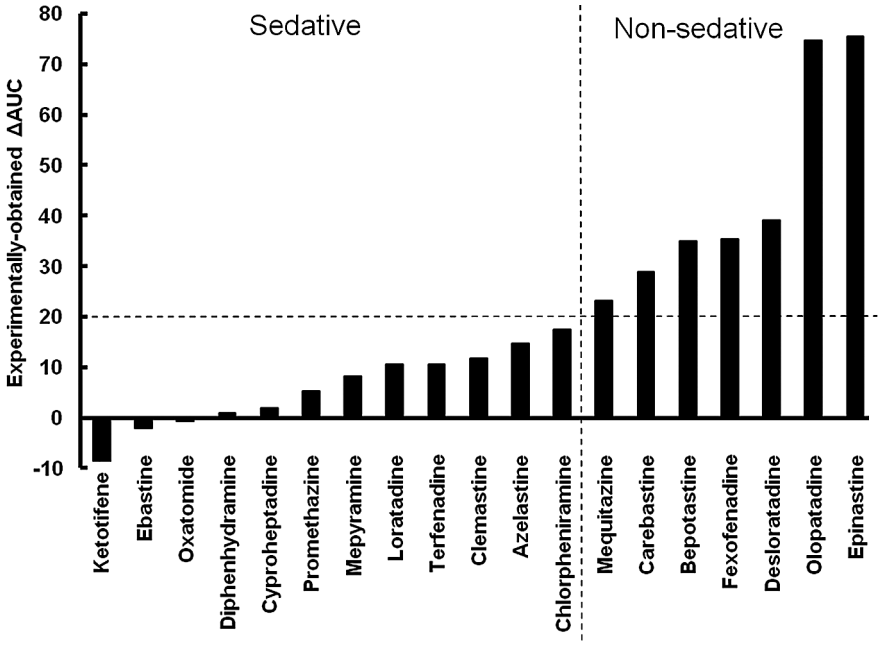

Fig. 4. Discrimination between sedative and non-sedative antihistamines in the training set by experimentally-obtained *Δ*AUC values. Sedative (left side) and non-sedative (right side) antihistamines were clearly distinguished by experimentally-obtained *Δ*AUC at an arbitrary value of 20.

no correlation between logD and IDDE was observed (VIF = 1). Regression diagnosis with the residual plot, plotting for the predicted *Δ*AUC value and the residual in the prediction of each antihistamine, revealed no specific abnormalities in the present model. More specifically, the residuals were almost normally distributed, and no skewness was found in the plot.

Using the above equation with a combination of logD and IDDE, sedative and non-sedative antihistamines in the external validation set were discriminated with 94% accuracy at the value of 20 arbitrary units of MRA-predicted *Δ*AUC (Fig. 6b, 15 of 16 antihistamines).

Discussion

Discrimination between sedative and non-sedative antihistamines by ΔAUC

*Δ*AUC values were evaluated as quantitative parameters to represent changes in the binding of antihistamines to H_1 receptors upon receptor internalization, which were considered to simply represent their membrane-penetrating ability (10). In the training set of antihistamines, sedative and non-sedative antihistamines were clearly discriminated by *Δ*AUC at the arbitrary unit of 20. Thus, *Δ*AUC appeared to be a very promising objective criterion for constructing a QSAR model to predict sedative and non-sedative properties of antihistamines.

Simple regression analyses

It is known that logD is one of the physicochemical descriptors related with the biomembrane permeability of drugs (19). Accordingly, of the various types of explanatory descriptors, the logD value was best correlated with *Δ*AUC; however, logD alone did not fully discriminate between sedative and non-sedative antihistamines in our assessment, as reported by others (20). This suggests that logD is important, but not adequate, for determination of the sedative and non-sedative properties of antihistamines; therefore, multiple regression models with plural descriptors were required to improve the predictability of the sedative effects of antihistamines.

Multiple regression analyses

The number of subjects is one of the most important factors to regulate the attribute number in multiple regression models because excess attribute numbers will likely result in a chance correlation. The number of attributes chosen in the construction process should be as small as possible in order to prevent a chance correlation. A systematic study suggested that the number of available descriptors in a multiple regression equation to maintain the predicting performance is 2, when the number of subjects is less than 20 compounds (21). Since the number of antihistamines in the training set was 19, the number of descriptors was restricted to 2 in this model. Variable selection was performed using the genetic algorithm approach, and the leave-one-out method was adopted as a validation system for the generalization capability of the model constructed (18). As a result, IDDE as well as logD were selected in the QSAR model. IDDE is a descriptor related to molecular shape, which is based on atomic networks in a molecule

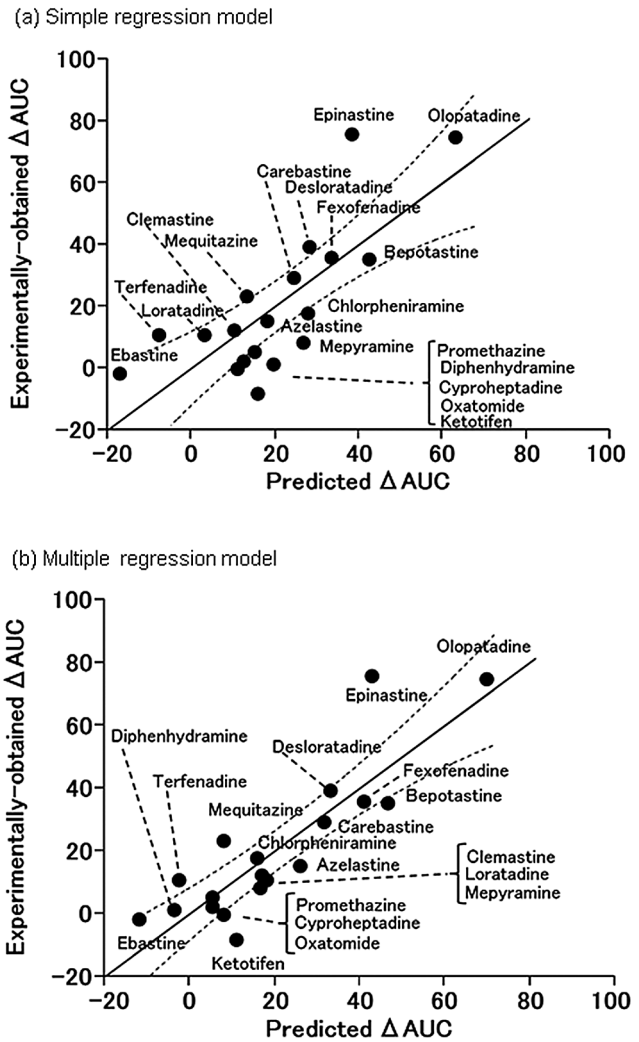

Fig. 5. Relationship between experimentally-obtained *Δ*AUC values and those predicted by the QSAR model constructed with simple (a) and multiple (b) regression analyses in the training set. Predicted *Δ*AUC were expressed as values obtained by the following equations:

Simple regression model: $\Delta AUC = -9.87\ logD + 44.2$

Multiple regression model: $\Delta AUC = -9.90\ logD + 23.0\ IDDE - 46.6$

In each plot, the solid line shows a linear least-squares fit and the dotted lines, the 95% confidence interval of the fit. Significant relationship was observed between *Δ*AUC values obtained experimentally and predicted by simple and multiple regression models with r^2 values of 0.584 and 0.742, respectively.

as one of the 2-dimensional descriptors calculated by Dragon (22):

$$IDDE = -\sum_{g=1}^{G} \frac{n_g}{A} \cdot \log_2 \frac{n_g}{A}$$

, where n_g is the cardinality of the gth set of vertices, G is the number of equivalence classes, and A the number of graph vertices. Although a simple regression equation between IDDE and *Δ*AUC was not statistically significant, the multiple regression equation with IDDE and logD indicated significance with a good determination coefficient (r^2) of 0.74. This finding of apparent improvement in the statistical property compared to the case of simple regression with logD or IDDE alone suggests that IDDE is an important parameter that enhances the prediction accuracy with logD. More specifically, it is presumed that spurious decorrelation between IDDE and *Δ*AUC was found by statistical adjustment of logD as a confounder.

Thus, the constructed multiple regression model achieved a high level of prediction performance for the external validation set of antihistamines. It is noted that the constructed model indicated non-sedative properties of dimetindene, although dimetindene is classified as a first-generation of antihistamine. In good accordance with this, there are evidences that dimetindene is as non-sedative as loratadine (23, 24). Furthermore, the constructed model well predicted non-sedative properties of bilastine (25), a newly-developed antihistamine, with a predicted *Δ*AUC value of 45.7 arbitrary units. Thus, the model is expected to have good generalization capability to predict sedative effects on a variety of seed compounds for antihistamines.

Simple diffusion as determinant of sedative and non-sedative properties of antihistamines

Since the model constructed is mainly based on extrapolations from in vitro studies on cells, there is a possibility that the predictability can be affected by factors such as transporters to regulate absorption, distribution, metabolism, and excretion of antihistamines in vivo. Actually, the rank order of sedative and non-sedative properties of antihistamines, which was evaluated by positron emission tomography (PET) by use of [^{11}C]doxepin in vivo (26), was not entirely identical but mostly compatible with our results: some discrepancies observed might be explained, at least in part, by the fact that the receptor occupancy by antihistamines in the brain varied according to their doses administrated (27), which resulted in changes in the rank order of their sedative and non-sedative properties in vivo (26). Thus, the assertion that sedative and non-sedative properties of antihistamines can be predominantly determined by their membrane-penetrating ability rather than their extrusion from the brain via P-glycoproteins is strengthened by the results that the QSAR model constructed on the basis of their membrane penetrating ability alone discriminated almost perfectly between sedative and non-sedative antihistamines. Furthermore, it is revealed that the two descriptors concerning their lipophilicity and molecular shapes, logD and IDDE, respectively, are involved in physicochemical properties

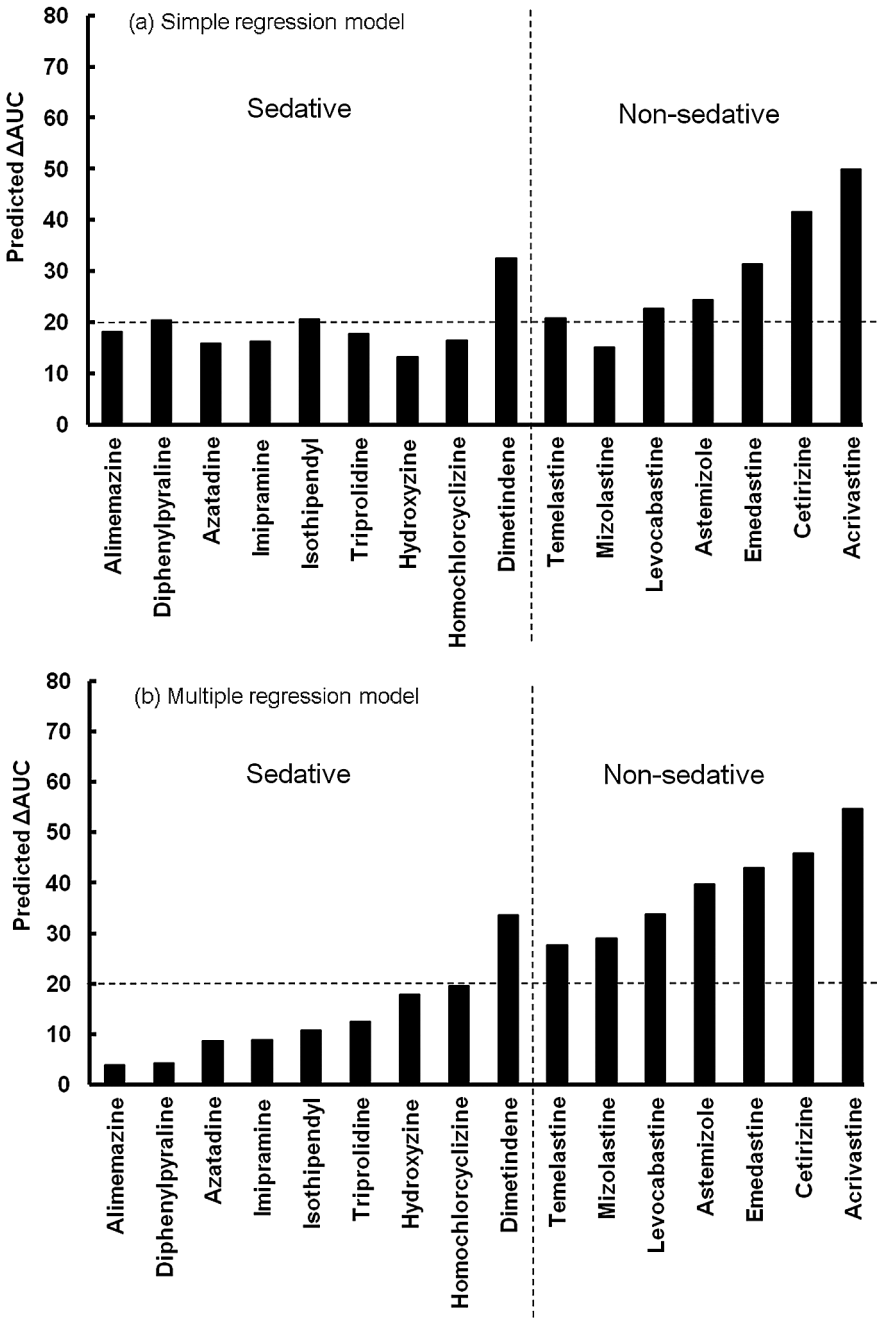

Fig. 6. Prediction of sedative and non-sedative antihistamines in the external validation set by the QSAR model constructed with simple (a) and multiple (b) regression analyses. a) Sedative (left side) and non-sedative (right side) antihistamines except diphenylpyraline, isothipendyl, dimetindene, and mizolastine were distinguished by predicted *Δ*AUC at an arbitrary value of 20. b) Sedative (left side) and non-sedative (right side) antihistamines except dimetindene were distinguished by predicted *Δ*AUC at an arbitrary value of 20.

of antihistamines to determine their membrane-penetrating ability for their side effects on the CNS.

Scope of application of this QSAR model

The prediction model was constructed for antihistamines in the training set. Although antihistamines have a wide range of diverse structures and physicochemical properties, the fundamental structure is to fit the binding cavity on H_1-receptor proteins. Accordingly, prediction for an external validation set of antihistamines with different chemical structures resulted in extremely good performance with the constructed model. Since it is generally considered that the QSAR prediction model should not be used for assessment using a deviant structure from the structural diversity used in the model construction, it appears that this prediction model might be restricted in application to compounds fitting the binding cavity on H_1 receptors.

Conclusion

We constructed a QSAR model to predict the sedative and non-sedative properties of antihistamines with high

accuracy, which indicated that molecular parameters concerning their lipophilicity and molecular shapes determines their membrane-penetrating ability for their side effects on the CNS. Although a variety of antihistamines have been developed so far, development of novel antihistamines such as bilastine is still in progress. Together with the recent findings of the crystal structure of the human H_1 receptor and differential binding sites of first and second generations of antihistamines responsible for their H_1-receptor specificity (28), the constructed QSAR model may contribute to develop novel or even further generation of antihistamines with increased specificity to H_1 receptors and reduced side effects on the CNS.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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