Risk Prediction Method for Anticholinergic Action Using Auto-quantitative Structure–Activity Relationship and Docking Study with Molecular Operating Environment

Materu Yuyama, Takeshi Ito, Yumiko Arai, Yuki Kadowaki, Natsumi Iiyama, Ayako Keino, Yurina Hiraoka, Takayuki Kanaya, Yasuyuki Momose, and Masaaki Kurihara

Graduate School of Pharmaceutical Sciences, International University of Health and Welfare Graduate School: 2600–1 Kitakanemaru, Ohtawara, Tochigi 324–8501, Japan: and Department of Pharmaceutical Sciences, International University of Health and Welfare; 2600–1 Kitakanemaru, Ohtawara, Tochigi 324–8501, Japan. Received March 17, 2020; accepted May 1, 2020

Lower urinary tract symptoms (LUTS) induced by anticholinergic drug action impair the QOL of patients and are associated with a poor prognosis. Therefore, it is expedient to develop methods of predicting the anticholinergic side effects of drugs, which we aimed to achieve in this study using a quantitative structure–activity relationship (QSAR) and docking study with molecular operations environment (MOE; Molecular Simulation Informatics Systems [MOLSIS], Inc.) In the QSAR simulation, the QSAR model built using the partial least squares regression (PLS) and genetic algorithm-multiple linear regression (GA-MLR) methods showed remarkable coefficient of determination ($R^2$) and $XR^2$ values. In the docking study, a specific relationship was identified between the adjusted docking score (-S) and bioactivity ($pKi$) values. In conclusion, the methods developed could be useful for in silico risk assessment of LUTS, and plans are potentially applicable to numerous drugs with anticholinergic activity that induce serious side effects, limiting their use.

Key words lower urinary tract symptom; anticholinergic action; in silico; quantitative structure–activity relationship; docking study

Introduction

Many drugs have anticholinergic actions as an unwanted side effect. For example, the antidepressant amitriptyline and anti-schizophrenic haloperidol are well-known for high incidences of anticholinergic effects. Lower urinary tract symptoms (LUTS) are some of the symptoms induced by anticholinergic drug action. LUTS, which is mediated by actions on the muscarinic M3 receptor, impairs the QOL of patients, leading to poor prognosis. In clinical practice, indications of risk for LUTS are required to be identified. However, in silico risk assessment methods for LUTS have not been established. Therefore, it is important to develop methods for the prediction of anticholinergic activity of drugs used clinically and those under development.

In silico quantitative structure–activity relationship (QSAR) and docking studies are available and generally used for screening promising drug candidates. In silico simulation requires a shorter estimation time than biological screening does. Moreover, QSAR and docking studies might be applicable to risk management and assessment of new and existing drugs. In this study, we developed methods for the prediction of anticholinergic actions of drugs using QSAR and docking studies. All computational simulations were carried out using molecular operations environment (MOE; Molecular Simulation Informatics Systems [MOLSIS] Inc.).

![Fig. 1. Typical Structures Used for Building Quantitative Structure–Activity Relationship (QSAR) Models](image-url)
Results and Discussion

Prediction by QSAR Model  The QSAR models were initially built based on the known activity values and structures of compounds and in this study, we used 82 compounds with known anticholinergic activity.23–29 We used binding affinity to the muscarinic M 3 receptor (pKi value) as the experimental value. In previous studies,23–29 the Ki value was calculated using IC 50. Therefore, the pKi value shows that binding affinity to the receptor is useful as an indicator of anticholinergic activity.23–29

Figure 1 shows the typical structures used for building the QSAR models. Subsequently, the calculated QSAR models were classified by the number of descriptors and as shown in Table 1, the coefficient of determination (R 2) tended to increase in a descriptor number-dependent manner.

Moreover, the R 2 value was approximately maximum when 13 descriptors were used30–32 in both the partial least squares (PLS) and genetic algorithm-multiple linear regression (GA-MLR) methods. Furthermore, a small difference between the R 2 and XR 2 value is generally desirable. Therefore, we adopted two QSAR models with 13 descriptors (Figs. 2A, 2B) and the QSAR models were further analyzed (PLS: R 2 = 0.808 and XR 2 = 0.696; GA-MLR: R 2 = 0.804 and XR 2 = 0.682.).

Table 1. Correlation of Determination in Quantitative Structure–Activity Relationship (QSAR) Models Calculated Using Partial Least Squares (PLS) and Genetic Algorithm-Multiple Linear Regression (GA-MLR)

| Number of descriptors | A. PLS | B. GA-MLR |
|-----------------------|--------|-----------|
| 12                    | R 2 = 0.793 | R 2 = 0.800 |
|                       | XR 2 = 0.670 | XR 2 = 0.685 |
| 13                    | R 2 = 0.808 | R 2 = 0.804 |
|                       | XR 2 = 0.696 | XR 2 = 0.682 |
| 14                    | R 2 = 0.811 | R 2 = 0.809 |
|                       | XR 2 = 0.659 | XR 2 = 0.671 |
| 15                    | R 2 = 0.812 | R 2 = 0.811 |
|                       | XR 2 = 0.649 | XR 2 = 0.656 |

Candidates of calculated QSAR models were classified by number of descriptors. When 13 descriptors were used, correlation of determination (R 2) score was large. Moreover, R 2 value was as large as that of XR 2.

Statistical Analysis of QSAR Model  The results of the
ANOVA showed that the QSAR models had a $p < 0.05$, suggesting that they could be considered statistically significant. To validate the accuracy of the QSAR models it was important to evaluate the multicollinearity and, therefore, this was statistically analyzed using two methods. One of methods involved evaluating the correlation between two descriptors and the other, evaluated the variance inflation factor (VIF) statistics. First, the correlation between two descriptors was calculated using the correlation matrix function of MOE (Figs. 3A, 3B), and values $>0.9$ (90%) indicated they could be considered to be strong relevant. In this method, multicollinearity might not occur in both QSAR models. Consequently, it was also necessary to evaluate the correlation between multiple descriptors because we used numerous descriptors. Therefore, secondary VIF statistics were calculated to evaluate the correlation between multiple descriptors using the IBM statistical package for the social sciences (SPSS) statistical program. A VIF statistics $>10$ is indicative of a strong relevant correlation between descriptors, and the result of the analysis showed that no VIF exceeded 10 for the QSAR models constructed using either the PLS or GA-MLR method. Thus, the PLS and GA-MLR QSAR models were both considered appropriate. In this study, we developed QSAR model using the PLS and GA-MLR methods to predict anticholinergic activity as follows: $R^2 = 0.808$, $XR^2 = 0.696$, $R^2 = 0.804$, $XR^2 = 0.682$, respectively.

**Docking Study**

Selection of Receptor Data

The muscarinic M₃ receptor, a G protein-coupled receptor and member of the seven-pass-transmembrane domain re-
ceptor superfamily, has been suggested to play an important role in the induction of LUTS.\(^ {1-7}\) Therefore, we used this receptor as the target protein in the docking study. There are six muscarinic M\(_3\) receptor types listed in the Protein Data Bank (PDB) and we selected the 5ZHP because it has the most recent publication year and muscarinic M\(_3\) receptor binding antagonist. Hydrogen addition, structural correction, and minimization energy were conducted with the 5ZHP. Redocking was performed for the preprocessed receptor using the 5ZHP ligand (Fig. 4). The result showed that an almost similar binding style was identified (root mean square deviation [RMSD] = 0.29 Å). However, advanced consideration of docking simulation conditions is required.

Setting Docking Simulation Conditions

In this procedure, 44 compounds already known to exhibit anticholinergic activity\(^ {24-29}\) were docked with the preprocessed 5ZHP (Fig. 5). Docking simulation was performed using the initial condition in the application and the scoring function was adopted as GB/VI. After 30 trials, the top five compounds with the highest docking scores (-S) were output.

Assessment of -S Value

Figure 6 shows the relationship between the adjusted -S and bioactivity (pKi) values depicted on the vertical and horizontal axes, respectively. The results of the docking study of compounds with already known anticholinergic activity revealed a specific relationship between the -S and pKi values. Therefore, this finding suggests that the docking study was suitable for predicting the anticholinergic activity of substances.

Conclusion

We developed methods for the prediction of anticholinergic actions of drugs using with QSAR and docking study simulations. In the QSAR simulation, the QSAR models constructed using PLS and GA-MLR methods showed impressive \(R^2\) and \(XR^2\) values; however, we discovered that evaluations of QSAR models should consider both \(R^2\) and \(XR^2\) values and VIF statistics.

The docking study demonstrated a specific relationship between the -S and bioactive value. Therefore, docking study could be available for prediction of anticholinergic activity. In conclusion, these developed methods could be useful for \textit{in silico} risk assessment of LUTS and plans are currently ongoing to apply these methods to several drugs, which may have anticholinergic action as serious side effects that limit their application. However, it is sometimes difficult to evaluate the prediction value, because information on drugs with anticholinergic action as the side effect is insufficient.
Experimental

Ligand structures were created using ChemDraw® (PerkinElmer, Inc.). These structures were properly preprocessed using MOE (Molecular Simulation Informatics Systems [MOLSYS] Inc.). The relevant data of the muscarinic M₃ receptor, SZHP, used as the target protein was downloaded from PDB and was properly preprocessed. The QSAR model construction and docking study were performed using MOE and the various statistical analyses were performed using MOE and IBM SPSS® statistical program.

QSAR Method

Building QSAR Model

QSAR models were calculated for 82 compounds with already known anticholinergic activity. The AutoQSAR function was adopted for the model building using both the PLS and GA-MLR regression models. PLS is a method for finding the optimal model by referring to the mechanism of biological evolution. Generally, it is possible to calculate a simpler and more accurate model using GA-MLR although it takes more time than the PLS method. Some conditions were considered in evaluating the constructed QSAR models. In particular, the difference between $R^2$ and $XR^2$ values was small, $R^2$ was large, and the QSAR models had smaller outliers.

Statistical Analysis of QSAR Models

Statistical analysis of the QSAR models was performed using an ANOVA and low urine tract symptoms. The correlation between descriptors was analyzed using the correlation matrix function (MOE) while the VIF was analyzed by the diagnosis of collinearity function (SPSS). The reference values used were 0.9 and 10, respectively.

Docking Study Method

Selection of Receptor in PDB

The PDB ID of SZHP, the muscarinic M₃ receptor, used as the target protein was identified. Hydrogen addition, energy minimization, and dummy site creation were conducted for SZHP before the docking simulation.

Setting Docking Simulation Conditions

The docking site was selected as the ligand binding site in SZHP, while initial settings were adopted for the other conditions. Docking simulation was performed for 44 compounds with already known anticholinergic activity. All compounds were identified from the literature.

Docking Study Evaluation

The docking simulation output used a five point ranking score (S1–S5) while accounting for the binding energy. We converted S to the -S value, which was considered the final docking score. Predicted and experimental values were plotted (Fig. 6).

Acknowledgments

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI (Grant nos. JP 16K08340).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Materials

The online version of this article contains supplementary materials.

References and Notes

1) Greenblatt D. J., Shader R. I., N. Engl. J. Med., 288, 1215–1219 (1993).
2) Abramis P., Andersson K. E., BJU Int., 100, 987–1006 (2007).
3) Iijima K.; Wachtler S. D.; Wyndaele J. J.; Eur. Urol., 52, 842–849 (2007).
4) Williams R. C., Chapple C. R., Yamanishi T., Yasuda K., Sellers D., J. J. Autoin. Pharmacol., 21, 243–248 (2001).
5) Yoshimura N., Neurourol. Urodon., 26 (Suppl.), 908–913 (2017).
6) Abraham N., Goldman H. B., Expert Opin. Pharmacother., 1, 79–93 (2015).
7) Kalisch Elleit L. M., Pratt L. N., Barratt J. D., Rowett D., Roughhead E., J. Am. Geriatr. Soc., 62, 690–695 (2014).
8) Takahashi S., Takei M., Nishizawa O., Yamaguchi O., Kato K., Gotoh M., Yoshimura Y., Takeyama M., Ozawa H., Shimada M., Yamanishi T., Yoshida M., Tomoe H., Yokoyama O., Koyama M., Low Urin Tract Symptoms, 8, 5–29 (2016).
9) Blais A. S., Nadeau G., Moore K., Genois L., Bolduc S., Eur. Urol., 52, 9–13 (2016).
10) Woodford H. J., Drugs Aging, 2018, 773–776 (2018).
11) Mitsui T., Takeda M., The Pharmaceuticals Monthly, 59, 69–75 (2017).
12) DuBeau C. E., J. Urol., 175 (3S), S11–S15 (2006).
13) Gnjidic D., Johnnell K., Clin. Exp. Pharmacol. Physiol., 40, 320–325 (2013).
14) Gnjidic D., Hilmer S. N., Blyth F. M., Naganathan V., Waite L., Seibelt M. J., McLachlan A. J., Cumming R. G., Handelsman D. J., Le Couteur D. G., J. Clin. Epidemiol., 65, 989–995 (2012).
15) Hashimoto M., Hashimoto K., Ando F., Kimura Y., Najase K., Arai K., J Pharm Health Care and Sci., 1, 7 (2015).
16) Coyne K. S., Sexton C. C., Kopp Z. S., Briton C. E., Milsom I., Chapple C., Br J. Urol., 108, 1459–1487 (2011).
17) Coyne K. S., Wein A. J., Tubaro A., Sexton C. C., Thompson C. L., Kopp Z. S., Ayer L. P., Br J. Urol., 103 (Suppl. 3), 4–11 (2009).
18) Kurihara M., Rowf A. S. S., Kansui H., Kagechiha H., Okuda H., Miyata N., Bioorg. Med. Chem. Lett., 14, 413–4134 (2004).
19) Hakamata W., Sato Y., Okuda H., Honzawa S., Saito N., Kishimoto S., Yamamoto A., Suguri T., Kittaka M., Kurihara M., Bioorg. Med. Chem. Lett., 18, 120–123 (2018).
20) Demizu Y., Nakatsu S., Sato Y., Honzawa S., Yamashita A., Suguri T., Kittaka A., Sato K., Okuda H., Kurihara M., Lett. Org. Chem., 8, 43–47 (2011).
21) Nagai J., Kagaya H., Uesawa Y., Chem-Bio Informatics Journal, 18, 1–9 (2018).
22) MOLSYS Inc., https://www.molsis.co.jp/, cited 19 February, 2020.
23) Peretto I., Forlani R., Fossati C., et al., J. Med. Chem., 50, 1571–1583 (2017).
24) Lee N. R., Zhang X., Dwoskin L. P., Zheng G., Bioorg. Med. Chem. Lett., 25, 5032–5035 (2015).
25) Sonoda S., Katayama K., Fujio M., Sakashtawa H., Inaba K., Asano K., Akira T., Bioorg. Med. Chem. Lett., 17, 925–931 (2007).
26) Lee M. J., Moon J. H., Lee H. K., Cho C. H., Choi S. H., Im W. B., Eur. J. Pharmacol., 843, 240–250 (2019).
27) Sykes D. A., Dowling M. R., Charlton S. J., Mol. Pharmacol., 76, 543–551 (2009).
28) Drug interview form of Eklira®, 5th edition, KYORIN Pharmaceutical Co., Ltd., 2018.
29) Drug interview form of ENCLUSE®, 5th edition, GlaxoSmithKline K.K., 2018.
30) Hou T. J., Xia K., Zhang W., Xu X. J., J. Chem. Inf. Comput. Sci., 44, 266–275 (2004).
31) Oprea Tudor I., J. Comput. Aided Mol. Des., 14, 251–264 (2000).
32) Kazius J., McGuire R., Bursi R., J. Med. Chem., 48, 312–320.
33) Wildman S. A., Crippen G. M., *J. Chem. Inf. Comput. Sci.*, **39**, 868–873 (1999).
34) Cooley W. W., Lohnes P. R., “Multivariate Data Analysis,” Wiley, New York, 1971.
35) Johnson J. W., *Multivariate Behav. Res.*, **35**, 1–19 (2000).
36) Seva U. L., Ferrando P. J., Chico E., *Behav. Res. Methods*, **42**, 29–35 (2010).
37) Gerber S. B., Finn K. V., “Using SPSS For Windows Data Analysis and Graphics,” Springer, Berlin, pp. 139–159, 2006.
38) Mansfield E. R., Helms B. P., *Am. Stat.*, **26**, 158–160 (1981).
39) Geladi P., Kowrski B. R., *Anal. Chim. Acta*, **185**, 1–17 (1986).
40) Riahi S., Ganjali M. R., Moghhadam A. B., Pourbasheer E., Norouzi P., *Curr. Anal. Chem.*, **5**, 42–47 (2009).
41) Arcos M. J., Alonso C., Ortiz M. C., *Electrochim. Acta*, **43**, 479–485 (1998).
42) Pourbasheer E., Riahi S., Ganjali M. R., Norouzi P., *J. Enzyme Inhib. Med. Chem.*, **25**, 844–855 (2010).