e-ISSN:2320-1215
p-ISSN:2322-0112

Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

A Modeling Approach towards Identifying Potential Bivalent Sensitizers of Neuromuscular Blocking Agents

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Research Article

Received date: 25/04/2016
Accepted date: 16/05/2016
Published date: 21/05/2016

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Keywords: Neuromuscular blocking agents, Sensitizers, Cross-sensitizers, Molecular modelling, Pharmacophore, Pholcodine, Chlorhexidine.

ABSTRACT

Objective: Anaphylactic reactions induced by neuromuscular blocking agents (NMBAs) can occur at first contact and might be due to cross-sensitization by other drugs or chemicals. Our aim was to investigate whether divalent molecules sharing chemical features with NMBAs might potentially cause cross-sensitization.

Methods: We constructed a pharmacophore key from chemical features common to all NMBAs (two positive or ionizable features 1.0807 nm apart) and used the key to screen FDA-approved small drug molecules of the Drug Bank® database (1541 molecules). The selected molecules were categorized on the basis of the values for three main parameters (fit value, relative energy and mean polar surface area).

Results: Screening from the pharmacophore key selected 13 NMBAs and 88 non-NMBA drugs. Of these 88 drugs, 42 had high-ranking parameter values and were considered preferential cross-sensitizers. These included the dopamine D2 receptor ligands aripiprazole and domperidone. Pholcodine, as well as nizatidine, ranitidine, antrafenine, cabergoline and, to some extent, chlorhexidine best fulfilled the required criteria of apolar character, bioavailability and ionization rate.

Conclusion: Our data support the hypothesis that pholcodine might be a potential NMBA cross-sensitizer. They confirmed the results of inhibition tests on patient serum suggesting that dopamine D2 receptor ligands might be cross-sensitizers. They also identified chlorhexidine, a widely used disinfectant incriminated in several cases of immediate hypersensitivity reactions, as a potential cross-sensitizer. Pharmacophore modelling is an inexpensive, straightforward approach that can be used to identify potential NMBA cross-sensitizing agents.

INTRODUCTION

Anaphylactic reactions during anesthesia are an issue of much concern and are caused mainly by neuromuscular blocking agents (NMBAs) through an immunoglobulin E (IgE)-mediated mechanism [1].

e-ISSN:2320-1215
p-ISSN:2322-0112

Anaphylactic reactions induced by NMBAs can occur at first contact and might be due to sensitization by contact with other drugs or chemicals. This problem is not specific to NMBAs since reactions to radio contrast media frequently occur on first exposure suggesting previous sensitization through an unknown molecule [2].

NMBAs belong to a variety of chemical families, all of which displaying two ammonium groups separated by a distance of 1-1.45 nm. These ammonium groups, which are either two quaternary ammonium ions (QAIs) or one QAI and a tertiary protonated amine, account for the ability to induce neuromuscular blockade [3] but are also responsible for NMBA binding to IgE. Two binding sites 1.5 nm apart may enable cross-linking of two mast cell-bound IgE molecules [4].

However, unlike most IgE-mediated allergic events, many NMBA-elicited reactions occur at first contact [4,5]. In addition, the NMBA-reactive IgE antibodies do not recognize just one NMBA but can recognize other NMBAs as well. This prompted the idea that some drugs and environmental agents might act as sensitizers and as inducers of IgE molecules able to bind NMBAs. Attempts to identify sensitizers having quaternary ammonium ions were initiated back in the 1980s in studies of the inhibition of binding to NMBA-specific IgE molecules using serum from allergic patients [6]. This approach identified mainly molecules with one (monovalent), two (divalent) or more quaternary ammonium groups but was limited by serum availability from allergic patients.

In our search for potential sensitizers in NMBA anaphylaxis we used a pharmacophore-oriented strategy which defines the chemical features constituting the common denominator of a set of molecules and which is widely used in drug discovery when no structural information on the biological target is available [7] and recently introduced to improve early detection of unexpected adverse effect [8]. A pharmacophore was generated from a representative set of NMBAs and a pool of small molecules was screened to identify putative NMBA sensitizing drugs.

METHODS

The pharmacophore generation protocol was developed using the Catalyst® algorithm based on the generalized CHARMm force field implemented in Accelrys Discovery Studio 2.5. **Figure 1** shows steps A or A' to D of ligand preparation, pharmacophore key generation and drug screening.

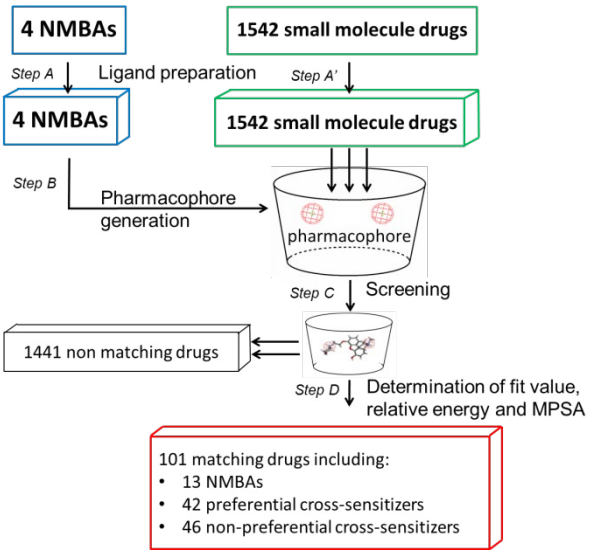

Figure 1. Pharmacophore key construction and drug screening procedure.

Ligand Preparation

Ligand preparation (step A or A') whether for pharmacophore key generation or drug screening involved: (i) ionization state attribution and generation of a 3-dimensional coordinate using the 'Prepare Ligands protocol'. Ionization state was predicted for a pH range of 5.5-9.5 using the estimated pKa values of the Drug Bank® database, (ii) generation of conformers using the *'Best'* option of the corresponding protocol for complete coverage of the conformational space [9].

Pharmacophore Generation

To design the pharmacophore, we superimposed four NMBAs (step B), each representing an NMBA class **(Figure 2)**: tubocurarine and atracurium, a rigid and flexible bistetrahydroisoquinoleine, respectively, succinylcholine, a flexible dimer of acetylcholine, and pancuronium, an amino-steroid. The 'HipHop' algorithm [10] of the 'Pharmacophore Generation protocol' produced a series of molecular alignments and identified the configuration of positive ionizable features or charges that were common to the molecules. The resulting pharmacophore key was composed of two positively charged or ionizable moieties with a mean between-centre distance of 1.081 nm (tolerance of 0.741-1.421 nm).

e-ISSN:2320-1215
p-ISSN:2322-0112

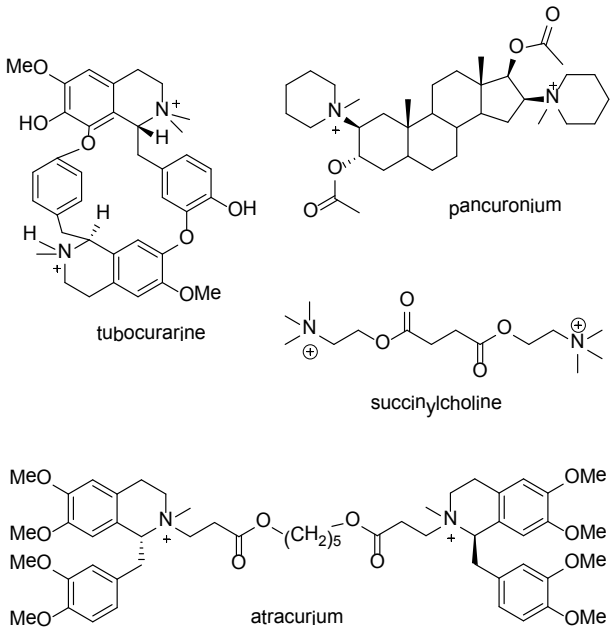

Figure 2. NMBAs used to construct the pharmacophore key.

Database Screening

We screened the Food and Drug Administration (FDA)-approved small molecule drugs of the Drug Bank® database (1541 drugs) [11] and inserted pholcodine to take into account current information involving this drug (pKa1=8.38; pKa2=6.67) [12] in the genesis of anaphylactic reactions [13,14]. We used the 'Pharmacophore Mapping protocol' (step D) with the 'flexible fitting' option to minimize the distances between the features of the pharmacophore key and to map atoms in the molecule. The matching parameters were fit value (FV), relative energy (RE) and mean polar surface area (MPSA). FV is a dimensionless parameter (range 0-2) describing the overlap between the chemical features of the pharmacophore key and conformational model. It is given by the equation $FV=\Sigma [1- \Sigma (D/T)^2]$ where D is the displacement of the feature from the centre of the location constraint and T is the radius of the location constraint sphere for the feature (aka tolerance). RE is the difference between the total energy and lowest available energy of a conformational model (range 0 to ∞, kcal.mol^{-1}). RE is related to the requirement for a sufficiently large population of conformers, which means that the relative population for the energy level is a function of RE. Molecules with a FV threshold above 1.5 and an RE threshold below 20 kcal.mol^{-1} were considered to have high-ranking parameter values. MPSA is the surface sum over all polar atoms of the molecule (Å^2) and was measured for each molecule to account for the apolar character of NMBAs. The inclusion of hydrophobic parameters which, unlike positive ionizable features or charges, are not well defined geometrically, could have given rise to false positives.

RESULTS

Figure 1 summarizes screening of the 1542 small molecule drugs of our database using the pharmacophore key that selected 101 drugs: 13 were NMBAs and 88 non-NMBAs. As an example, **Figure 3** shows pholcodine fitting in the pharmacophore key.

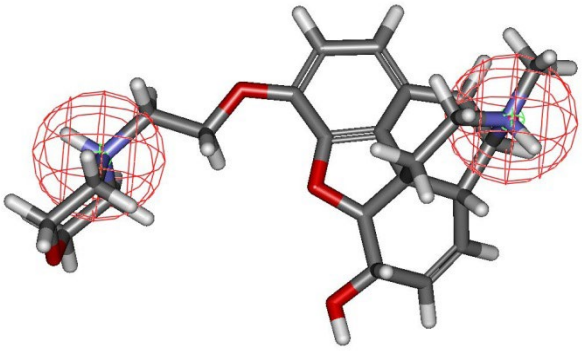

Figure 3. Pholcodine fit within the pharmacophore key: positive ionizable features or charges (red sphere) pholcodine presented as sticks with carbon (grey), hydrogen (white), oxygen (red) and nitrogen (blue).

Two entries are given for each NMBA in **Table 1**: i- the best FV and associated RE, ii- the lowest RE and associated FV.

e-ISSN:2320-1215
p-ISSN:2322-0112

All 13 NMBAs had either a high FV or low RE. The large number of NMBAs, selected with our pharmacophore key, including hexafluoronium (a nicotinic acetylcholine receptor antagonist), indicated that this key was indeed the common denominator of this class of drugs. Our pharmacophore key robustness was verified by the following: (i) we twice randomly selected four NMBAs other than those of **Figure 2** to obtain two other pharmacophore whose moieties were separated by a distance of 0.9094 and 1.2293 nm. Of the three pharmacophore, our initial pharmacophore key had the best average FV for all NMBAs (1.95 versus 1.88 and 1.78 for the two others); (ii) we changed one NMBA among our four chosen NMBAs with an NMBA of the same class. The distance between the two moieties was invariable (1.081 nm). The only NMBA of our database that was not selected was pipecuronium because of its particular ionization profile.

Table 1. Parameter values for NMBA fit in the Pharmacophore.

	By best FV		By lowest RE		MPSA[c]
	FV[a]	RE[b]	FV[a]	RE[b]	
Alcuronium	1.828	12.575	1.594	0.192	46.94
Atracurium	1.999	14.71	1.998	10.55	126.4
Decamethonium	2	7.138	1.999	2.737	0
Doxacurium	1.990	11.50	1.924	1.600	163.4
Gallamine	1.990	6.824	1.999	1.714	41.01
Hexafluoronium	1.58	12.0149	1.5703	5.8691	0
Metocurine	1.998	3.128	1.991	1.291	55.38
Mivacurium	1.999	0.1314	1.999	0.08426	144.9
Pancuronium	1.959	8.291	1.958	2.159	52.60
Rocuronium	1.999	3.202	1.975	1.016	60.20
Succinylcholine	2	3.832	1.999	3.597	52.60
Tubocurarine	1.999	13.01	1.995	1.39 10^{-14}	84.65
Vecuronium	1.999	5.707	1.990	1.39 10^{-14}	57.04

[a]FV, fit value; [b]RE, relative energy (kcal.mol^{-1}); [c]MPSA, mean polar surface area (Å^2)

Among the 88 selected non-NMBA drugs, 42 were considered to be "preferential sensitizers" because of their high-ranking FV and RE matching parameter values **(Table 2)**.

Table 2. Parameter values for the 42 non-NMBA drugs considered to be preferential sensitizers.

		By best FV		By lowest RE		MPSA[c]
		FV[a]	RE[b]	FV[a]	RE[b]	Å^2
Cough suppressant	Pholcodine[e]	1.991	8.875	1.983	5.739	56.80
Acetylcholinesterase inhibitors	Ambenomium	1.999	3.972	1.999	2.189	58.20
	Demecarium	1.999	3.682	1.999	3.682	59.08
NMBA potentiators (Antibiotics)						
Aminoglycosides	Amikacin	1.999	6.794	1.999	4.60 10^{-4}	336.8
	Arbekacin	2	11.77	1.999	0.04728	302.1
	Framycetin	2	3.367	1.999	8.6 10^{-4}	362.8
	Gentamicin	2	9.091	1.999	7.2 10^{-3}	212.1
	Kanamycin	1.999	15.73	0.5330	2.2 10^{-13}	289.1
	Netilmicin	1.999	20.90	1.999	0.7686	213.7
	Paromomycin	2	21.06	1.999	0.1086	355.4
	Tobramycin	1.999	21.31	1.907	1.0 10^{-14}	274.6
Polypeptides	Colistimethate	1.999	10.62	0.8889	5.39E-14	771.5
	Colistin	2	5.47E-05	0.5713	-7.30E-14	498.7
	Polymyxin B	1.999	8.812	1.611	-2.09E-14	495.5
Tetracyclines	Lymecycline	2	8.042	0.3496	3.840	261.7
	Tigecycline	1.999	16.22	1.530	1.39 10^{-14}	215.6
Other categories						
Macrolides (antibiotics)	Azithromycin	1.999	17.26	1.999	4.327	182.5
	Dirithromycin	1.999	7.30.10^{-06}	1.999	7.3 10^{-06}	202.1
Other antibiotics	Quinupristin	1.999	20.79	1.983	2.07E+00	258.9
	Vancomycin	1.641	8.566	1.641	8.566	539.5
Antineoplastics	Imatinib	2	8.326	1.999	5.598	92.06
	Mitoxantrone	1.999	7.037	1.999	1.445	172.3
	Porfimer	1.999	4.787	1.999	4.787	316.9
	Vinorelbine	1.995	11.20	1.868	2.987	136.3

e-ISSN:2320-1215
p-ISSN:2322-0112

H_2 antagonists	Nizatidine	2	6.581	1.999	3.056	149.9
	Ranitidine	2	11.42	1.999	4.004	136.5
Antipsychotics	Pipazethate	1.882	15.71	1.831	2.248	82.60
[d]D_2 ligand	Aripiprazole	1.999	1.925	50.59	1.729	50.59
D_2 ligand	Domperidone	2	4.976	2	4.976	74.90
Beta blockers	Carteolol	1.964	8.272	1.860	0.5167	79.75
	Carvedilol	1.871	18.32	1.850	8.278	81.14
Miscellaneous	Antrafenine	1.999	30.02	1.998	5.486	64.68
	Cabergoline	1.911	22.02	1.793	6.195	74.08
	Caspofungin	1.999	16.74	1.998	2.361	418.2
	Chlorhexidine	1.999	11.77	1.999	6.094	178.5
	Colestipol	2	2.221	1.999	1.780	105.1
	Deferoxamine	1.999	1.207	2	5.546	211.1
	Plerixafor	2	2.543	1.999	$3.1\ 10^{-4}$	108.5
	Spermine	1.999	3.273	1.999	2.022	88.50
	Degarelix	1.999	14.34	1.120	0	526.6
	Terlipressin	1.999	7.218	1.338	0.07420	566.7
	Vapreotide	1.999	16.44	1.999	1.157	404.5

[a]FV, fit value; [b]RE, relative energy (kcal.mol^{-1}); [c]MPSA, mean polar surface area (Å^2); [d]D2: dopamine receptor D2; [e]In bold characters, the 5 molecules with best fit parameters (FV, RE, MPSA and ionization rate)

Many of these 42 drugs were to be expected on account of their pharmacologic properties (the acetylcholinesterase inhibitors ambenonium and demecarium) or on account of their action as NMBA potentiators (8 aminoglycosides, 3 polypeptides and 2 tetracycline's). In addition, there were 4 antibiotics (2 macrolides, 1 streptogramin (quinupristin), 1 glycopeptide (vancomycin)), 4 antineoplastic, 2H2 antagonists, 3 antipsychotics of which 2 were ligands of the dopamine D2 receptor (aripiprazole and domperidone), 2 beta blockers and 11 miscellaneous drugs. Some were less likely "preferential sensitizers" because they had a higher MPSA than the NMBAs. Selected drugs with a high MPSA were all the antibiotics, including those known as NMBA potentiators, and 4 miscellaneous drugs (deferoxamine, degarelix, terlipressin and vapreotide). The drugs with the best fitting parameters in terms of FV, RE, MPSA and ionization state for our pharmacophore were nizatidine, ranitidine, antrafenine, cabergoline and pholcodine. The remaining 46 drugs were denoted "non-preferential sensitizers" **(Table 3).** They included 22 iminium compounds with a planar structure in stark contrast to the tetrahedral structure of a QAI, 16 drugs with low matching parameters (FV<1.5 and/or a RE>20 kcal.mol^{-1}), 5 ligands of gadolinium the conformers of which had been generated without gadolinium and 3 ubiquitous amino-acids (L-Arg is also an iminium compound).

Table 3. Parameter values for the 46 non-NMBA drugs considered to be non-preferential sensitizers.

	By best FV		By lowest RE	
	FV[a]	RE[b]	FV[a]	RE[b]
Iminiums				
Azathioprine	0.9447	0.9438	0.6047	-7.133 E-04
Bacitracin	1.999	22.53	1.062	-2.25E-04
Capreomycin	2	14.22	1.999	4.68E+00
Cimetidine	1.981	12.78	1.901	8.778
Chloroquine	1.999	7.880	1.986	5.951
Eszopiclone	1.832	23.94	1.715	6.816
Famotidine	1.999	7.13	1.999	3.994
Gefitinib	1.997	12.53	1.982	3.612
Gonadorelin	1.999	11.19	1.982	4.909
Icatibant	2	13.73	1.999	0.4263
Irinotecan	1.999	8.599	1.999	4.655
Hydroxychloroquine	1.999	12.42	1.995	7.288
Hydroxystilbamidine	1.999	5.191	1.999	2.560
Nafarelin	1.999	30.44	0.8733	12.37
Pentamidine	1.999	6.914	1.999	6.246
Proflavine	1.865	0	1.865	0
Quinacrine	1.996	5.446	1.9618	4.163
Streptomycin	1.999	13.57	1.333	-4.17E-14
Valganciclovir	1.999	12.04	1.999	5.935
Valaciclovir	1.999	7.825	1.999	6.200
Vardenafil	2	5.168	1.999	3.381
Zopiclone	1.882	32.54	1.739	12.14
Drugs with low matching parameter values				

Acetophenazine	0.781821	16.29881	0.677224	4.30114
Bleomycin	1.993296	24.58758	1.18941	24.5544
Carphenazine	0.732206	11.04386	0.701733	6.762175
Dimethindene	1.017	20.8	0.9254	10.20
Fluphenazine	0.702513	11.46592	0.6800079981	3.49333
Lisdexamfetamine	0.5941	9.593	0.4764	7.183
Lisinopril	0.466323	11.67692	0.4337678	5.053228
Pentolinium	0.847707	12.504957	0.686721	5.4655
Perphenazine	0.700605	3.378792	1.00329	14.18785
Prochlorperazine	0.732011	3.51842	0.732011	3.51842
Rolitetracycline	1.3435	14.807999	1.27541	6.36621
Ropinirole	0.366417	14.65474	0.2801067	11.172
Sitagliptine	0.6601	8.654	0.5431	5.182
Thioproperazine	0.7542167	14.3766	0.698052	5.4121
Triethylperazine	0.77373	16.33269	0.675515	4.77848
Trifluoperazine	0.766536	9.2605956	0.697729	3.40221
Gadolinium contrast agents				
Gadobenate Demiglumine	0.3514	31.49	0.3250	11.90
Gadodiamide	0.4107	10.94	0.3822	9.465
Gadofosveset Trisodium	0.3916	28.77	0.2435	5.304
Gadopentate Meglumine	0.5390	22.24	0.4187	9.280
Gadoversetamide	0.4373	18.35	0.3247	12.27
Aminoacids				
L-arginine	1.423272	9.116566	1.30116	3.84341
L-cystine	1.867698	10.49869	1.83217	7.21531
L-lysine	0.795507	9.87638	0.464607	1.95225

[a]FV, fit value; [b]RE, relative energy (kcal.mol^{-1})

DISCUSSION

Screening using our pharmacophore key identified 13 NMBAs among 14 NMBAs included small molecule drugs of our data base, thereby confirming that the key was the common denominator of the NMBA structure. The key also identified 88 non-NMBAs. Among these were 42 "preferential sensitizers", selected on the basis of either a high drug-key overlap (FV>1.5) or a low energy needed to fit the key (RE <20 kcal.mol^{-1}). The drug's MPSA was also considered in order to account for the apolar character of NMBAs apart from the two ammonium centers. The 42 "preferential sensitizers" included antibiotics, antineoplastic, H2 antagonists, antipsychotics, beta blockers, as well as pholcodine, chlorhexidine, antrafenine, cabergoline, caspofungin, colestipol, deferoxamisome, plerixafor and even spermine.

The presence of chlorhexidine and pholcodine among the 42 non-NMBAs drugs is worthy of interest. Regarding pholcodine, because it could contribute to the hypothesis of its role as a sensitizer in anaphylactic reactions during anesthesia with NMBAs [13-15] and chlorhexidine, because this drug is both a disinfectant for external use as an antiseptic coated in central venous catheter [16].

Among the selected drugs with high bioavailability, some had two basic amines (e.g. nizatidine, ranitidine, antrafenine, cabergoline and pholcodine) and consequently a significant ionization rate [12], thereby meeting all criteria. The anti-psychotics pipazethate and the two dopamine D2 receptor ligands aripiprazole and domperidone, as well as the beta-blockers carteolol and carvedilol, with at least one weak basic function, have a reduced ionization rate and are therefore less likely to bind.

Some of our findings are quite close to those of Baldo [4] who performed inhibition assays in two subjects with anaphylactic reaction to alcuronium and identified pentolinium and the dopamine D2 receptor ligands, promethazine and chlorpromazine, as cross-reacting molecules. We also identified two dopamine D2 receptor ligands (aripiprazole and domperidone) as well as pentolinium, but it was low ranking.

In conclusion, this inexpensive, straightforward molecular approach proved to be an effective tool in the preliminary identification of potential NMBA sensitizing agents. Our pharmacophore key identified 88 putative NMBA sensitizers of which 42 had high-ranking parameter values and 5/42 fulfilled all criteria, namely, pholcodine, nizatidine, ranitidine, antrafenine and cabergoline. Chlorhexidine, also listed among the 42 is of special interest.

There is no *in vitro* method to identify cross sensitizers and until now cross sensitizers identification are based on clinical observations. Screening for candidate sensitizing drugs by computational analysis may be a first step and help clinicians toward identifying drugs with strong sensitizing potential.

e-ISSN:2320-1215
p-ISSN:2322-0112

REFERENCES

1. Mertes PM, et al. Anaphylaxis during anesthesia in France: an 8-year national survey. J Allergy Clin Immunol 2011;128:366-373.
2. Lerondeau B, et al. Analysis of cross-reactivity among radio contrast media in 97 hypersensitivity reactions. J Allergy Clin Immunol 2015.
3. Cheymol J. Synthetic inhibitors of neuromuscular transmission, chemical structures and structure-activity relationships. Neuromuscular Blocking and Stimulating Agents. Pergamon Press, Oxford. 1972;243-294.
4. Baldo BA and Fisher MM. Anaphylaxis to muscle relaxant drugs: cross-reactivity and molecular basis of binding of IgE antibodies detected by radioimmunoassay. Mol Immunol. 1983;20:1393-1400.
5. Fisher MM and Munro I. Life-threatening anaphylactoid reactions to muscle relaxants. Anesth Analg. 1983;62:559-564.
6. Baldo BA, et al. on the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: an immunochemical perspective. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2009;39:325-344.
7. Leach AR, Gillet VJ, Lewis RA, Taylor R. Three-dimensional pharmacophore methods in drug discovery. J Med Chem. 2010;53:539-558.
8. Vilar S, et al. 3D pharmacophoric similarity improves multi adverse drug event identification in Pharmacovigilance. Sci Rep. 2015;5:8809.
9. Catalyst User Guide. Accelrys Software Inc, San Diego. 2005.
10. Clement O and Mehl A. Pharmacophores based on multiple common-feature alignments. Pharmacophore Perception, Development, and Use in Drug Design. Guner OF, La Jolla. 2000;71-84.
11. Wishart DS, et al. Drug Bank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res. 2008;36:D901-906.
12. Kovacs Z, et al. (2006) Site-specific acid-base properties of pholcodine and related compounds. Anal Bioanal Chem 386:1709-1716.
13. Johansson SGO, et al. Pholcodine caused anaphylaxis in Sweden 30 years ago. Allergy. 2009;64:820-821.
14. Katelaris CH, et al. Pholcodine consumption and immunoglobulin E-sensitization in atopic from Australia, Korea, and Japan. Asia Pac Allergy. 2014;4:86-90.
15. Florvaag E, et al. Pholcodine stimulates a dramatic increase of IgE in IgE-sensitized individuals. A pilot study. Allergy. 2006;61:49-55.
16. Beaudouin E, et al. Immediate hypersensitivity to chlorhexidine: literature review. Eur Ann Allergy Clin Immunol. 2004;36:123-126.