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Structure–activity relationships and computer-assisted analysis of respiratory sensitization potential

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Abstract

The mechanism(s) underlying respiratory sensitivity to chemicals is uncertain but is assumed to involve immunologic components with pharmacologic and neurologic involvement. Predictive testing would be valuable to prevent occurrence of hypersensitivity. Several in vitro and in vivo approaches have been used for predictive purposes. In vitro methods have included assessment of the ability of the chemical to undergo reaction with proteins. Computational methods have investigated the relationship between structure and electrophilic potential of chemical allergens. We have initiated a structure–activity evaluation of chemicals associated with elicitation of respiratory sensitization and have utilized a computer-based expert system, MultiCASE. A preliminary database of 39 active chemicals has been established from a literature search of clinical case reports and animal test results. Evaluation of the model has indicated structural alerts for activity which consist of structural fragments as well as physicochemical properties. Further development of the model and evaluation of findings should enable mechanistic insight into the process of respiratory sensitization and recognition of factors which distinguish respiratory sensitizers mechanistically from other chemical allergens such as contact sensitizing chemicals.

Keywords: Respiratory sensitivity; Structure–activity relationship (SAR); MultiCASE; Structural alerts; Predictive model

1. Introduction

Respiratory allergy, including rhinitis, hypersensitivity pneumonitis, and asthma, has been recognized in association with numerous airborne agents. Most frequently these agents are of high molecular weight (HMW), i.e. proteins larger than 10 kDa. Many of these allergens possess biological activity; for example, some proteases and lipases are recognized as potent respiratory allergens. In contrast, only a limited number of low molecular weight (LMW) chemicals have been associated with respiratory allergy. A further distinction between HMW and LMW allergens is apparent when one considers the demographics of sensitized populations. Whereas a large percentage of individuals with sensitivity to HMW allergens are atopic, only 25% of those with hypersensitivity to LMW chemicals are atopic.

Respiratory hypersensitivity can be manifested

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by acute response to the offending allergen, i.e. immediate-onset responses (IAR), or those which occur hours later, i.e. late-onset reactions (LAR). HMW allergens elicit predominantly IAR, whereas LMW allergens frequently produce LAR. This distinction has implications for genetic evaluations and for predictive testing since progress has been made in elucidation of the genetic control of IgE production. Clearly, mechanistic information for both IAR and LAR should guide future development of predictive tests for chemical respiratory allergens.

Since respiratory allergens comprise diverse industrial and consumer products, the need for predictive tests is apparent. Several in vitro and in vivo models have been developed [1], but validation has been limited. There is a need for an objective structure–activity relationship (SAR) model to reduce the reliance on animal testing and provide insight into mechanistic processes. Such a SAR model is under development in this laboratory.

Several in vitro methods have been described for predicting respiratory sensitizing properties of chemicals. Wass and Belin [2] employed high-pressure liquid chromatography to monitor reactivity of suspect chemical allergens with a lysine-containing peptide. Reaction conditions included aqueous solution at neutral pH and 37°C. Binding was noted with three chemicals recognized as respiratory sensitizers, and with chemicals which have not been classified as respiratory sensitizers (i.e. with isobutyl chloroformate). Such a model will need to address factors such as solubility and metabolism. It is unclear how this test could distinguish contact from respiratory sensitizers since both groups of agents are known to be chemically reactive toward nucleophiles.

A tier approach to the evaluation of the potential of low molecular weight chemicals for causing respiratory hypersensitivity has been proposed [3]. The first tier is a structural examination of the chemical to assess its potential for covalent reaction with protein. The process involves a search of the literature for information on immunogenic effects of the chemical, or structurally-related compounds, in humans or in animals. The second tier is an in vitro test of the ability of the chemical to react with proteins. A variety of conditions are used to effect coupling including a range of concentrations of the chemical, variations in pH and in temperature and time of incubation. If positive results are obtained in tiers 1 and 2, the chemical is evaluated in tier 3 which involves in vivo testing.

2. Quantitative structure–activity relationship (QSAR)

The widespread occurrence of respiratory hypersensitivity and the current reliance on animal testing to identify sensitizing chemicals indicates a need to develop alternative quantitative methodologies. A structure–activity relationship (SAR) model could fulfill this need and provide mechanistic information as well. SAR models exist for many other toxic endpoints including carcinogenicity, mutagenicity, reproductive toxicity and contact sensitivity. However, no model exists for respiratory hypersensitivity.

2.1. MultiCASE

The Multiple Computer Automated Structure Evaluation (MultiCASE) system [4] operates by fragmenting all chemicals in a database into substructures containing two or more heavy (non-hydrogen) atoms. It then identifies fragments which are statistically associated with activity. This system differs from other SAR models by not assuming a mechanism of toxicity. MultiCASE is therefore ideal for prediction of chemicals causing respiratory hypersensitivity, a toxicity with an uncertain mechanism.

2.2. QSAR for contact sensitivity

Several models have been developed for contact sensitivity, and are listed in Table 1. A commonality among these systems is that their development was based on a hypothesized mechanism of sensitization, i.e., the absorption of the chemical sensitizer through the skin followed by its covalent modification of a protein. Each model proposed structural alerts which, in all cases, comprised electrophilic moieties, or moieties which can be

Table 1
Comparison of QSAR models for contact sensitization (from [5])

Method	Size	Source	Structural alerts	Physico-chemical properties	Sensitivity	Specificity	Reference
Derek	294	GPMT	Protein reactive[a]	Skin penetration	98%	82%	[6]
Derek	93	Various animal tests	Protein reactive[a]	Skin penetration	58%	92%	[7]
RAI		Various animal tests	Protein alkylation	Log *P*	Not applicable	Not applicable	[8]
PROPHET	2200	Reports in contact dermatitis	Protein reactive[a]	None	Not available	Not available	[9]
Classification model of ACD	72	Database of Ziegler	Protein binding, molar refraction, H binding	Log *P*	79%	88%	[10]
MultiCASE	1034	GPMT HMT Human patch	Computer-generated	Log *P*, HOMO, LUMO, MWt, Water sol.	78%	95.6%	[5]

[a]Assumed mechanism of reaction includes binding of chemical sensitizer to a protein.
Abbreviations: GPMT, guinea pig maximization test; HMT, human maximization test; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; MWt, molecular weight; water sol., water solubility.

metabolized into electrophilic fragments (pro-electrophiles). We employed MultiCASE to evaluate a database of contact sensitizers and identified structural alerts associated with sensitization [5]. The database was derived from reports of animal and human studies and consisted of 1034 chemicals of which 317 were classified as active, 22 had marginal (questionable) activity, and 695 were inactive.

MultiCASE identified 49 biophores (fragments which are statistically associated with activity). The major biophores were: (1) a nitrogen double bonded to a carbon or a nitrogen, (2) substituted aromatic structures, (3) thiol and disulfide containing fragments, and (4) electrophilic moieties. Comparison of the predicted activity of chemicals not in the learning set, with experimental results, yielded a concordance of 90%.

2.3. QSAR for respiratory hypersensitivity

A database of LMW chemical sensitizers was established from a critical review of the published literature. Criteria were established for accepting reports of active chemicals and are presented in Table 2. The criteria were analogous to those which we established when developing our database of contact sensitizers. Emphasis was placed on the elicitation phase of the hypersensitivity response. Human case reports as well as animal studies were used to identify chemical asthmagens. For human cases, we required that the chemical cause a decrease of $\geq 20\%$ in FEV_1 upon provocation challenge, with response occurring within 24 h of challenge. For animal studies, a

Table 2
Respiratory sensitizers[a]: criteria for acceptance of data

A. Clinical studies
Inhalation challenge with chemical
Non-irritating concentration of chemical used
Response is decrease in $FEV_1 \geq 20\%$ within 24 h

B. Animal studies
Inhalation challenge with chemical
Non-irritating concentration of chemical used
Response is a statistically significant decrease in lung function (relative to baseline, or to control animals)
Adjuvant may be used for induction, but not for challenge

[a]Chemicals must be ≤ 1 kDa.

response to inhalation challenge was required which exceeded 3 standard deviations from the mean response of control animals. A total of 39 chemicals was identified from the literature search. As indicated in Table 3, these chemicals included diisocyanates, acid anhydrides, dyes and others.

A problem arose when compiling a list of inactive chemicals, i.e. those shown not to cause sensitization. Because of the absence of reports of inactive chemicals (except for lactose), the assumption was made that a chemical which did not cause contact sensitivity would also be inactive as a respiratory sensitizer. Accordingly, 39 chemicals known to be inactive for causing dermal sensitivity were included in the MultiCASE learning set.

A preliminary test of the model was conducted

Table 3
Respiratory chemical allergens

Isocyanates	**Dyes**
Diphenylmethane-4,4′-diisocyanate (MDI)	Brilliant orange GR
Hexamethylene diisocyanate (HDI)	Carminic acid
Isophorone diisocyanate (IPDI)	Reactive orange 3R
Naphthalene-1,5-diisocyanate	Rifafix red BBN
Toluene 2,4-diisocyanate (2,4 TDI)	Rifazol black GR
Toluene 2,6-diisocyanate (2,6 TDI)	
Amines	**Acid anhydrides**
Dimethyl ethanolamine	Phthalic anhydride
Ethanolamine	Tetrachlorophthalic anhydride
Ethylenediamine	Trimellitic anhydride
Triethylenetetramine	
Others	**Others**
Abietic acid	Glutaraldehyde
6-Aminopenicillanic acid	Iso-nonanoyl sulphonate oxybenzene
7-aminocephalosporanic acid	Methyl-2-cyanoacrylate
Ampicillin	a-Methyldopa
Azocarbonamide	Phenylglycine acid chloride
2-(*N*-Benzyl-N-*tert*-butylamino)4′-hydroxy-3′-hydroxymethylacetophenone diacetate	Piperacillin
	Piperazine
	Plicatic acid
Benzylpenicillin	Spiramycin
Cephalexin	Styrene
Chlorhexidine	Tylosin
Ethyl cyanoacrylate	

Table 4
Mean physico-chemical properties of respiratory sensitizers

Property	Active chemicals (mean ± S.D.) (n = 39)	Inactive chemicals (mean ± S.D.) (n = 39)	P values
Mol. wt.	319 ± 241	191 ± 50.0	0.0016
HOMO	0.780 ± 0.32	1.10 ± 0.63	0.006
LUMO	−0.547 ± 0.71	−1.01 ± 0.63	0.003
(HOMO − LUMO)/2	0.116 ± 0.27	0.0467 ± 0.17	0.185
Log P	2.22 ± 2.7	3.44 ± 1.0	0.013
Water solubility	1.12 ± 2.8	−0.668 ± 1.45	0.0007

Abbreviations: n, number of chemicals; mol. wt., molecular weight; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; log P, octanol/water partition coefficient; water solubility = log S where S is g/100 g water.

to determine whether certain physicochemical characteristics were associated with respiratory sensitizers. As is evident from Table 4, active chemicals were associated with higher molecular weight, lower log P values, and greater water solubility. In addition, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies differed between the two groups of chemicals.

In order to probe mechanistic differences between respiratory and dermal sensitizers, the physicochemical properties of the two groups of chemicals were compared. Several significant differences were noted, as indicated in Table 5. Res-

Table 5
Mean physico-chemical properties of respiratory vs. contact sensitizers

Property	Respiratory chemicals (mean ± S.D.) (n = 39)	Contact chemicals (mean ± S.D.) (n = 317)	P values
Mol. wt.	319 ± 241	216 ± 112	0.000005
HOMO	0.780 ± 0.33	0.760 ± 0.33	0.730
LUMO	−0.547 ± 0.71	−0.723 ± 0.50	0.049
(HOMO − LUMO)/2	0.116 ± 0.27	0.0187 ± 0.24	0.019
Log P	2.22 ± 2.8	2.64 ± 2.14	0.275
Water solubility	1.12 ± 2.8	0.147 ± 2.4	0.0187

Abbreviations as in Table 4.

piratory chemicals had higher mean molecular weight and greater water solubility when compared with dermal sensitizers.

3. Conclusions

Several methods are available to predict the respiratory sensitizing potential of chemicals. Each method has distinct advantages and disadvantages. Our QSAR model is in the initial stages of development. Preliminary studies indicate that the model can distinguish properties associated with respiratory sensitizers and contrast them with those causing dermal sensitivity. The model remains to be validated using known respiratory sensitizers. It is anticipated that use of the model will not only allow prediction of activity of untested chemicals, but also provide insight into mechanisms underlying respiratory hypersensitivity.

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