Exploring the Relationship of Drug-Induced Neutrophil Immaturity & Haematological Toxicity to Drug Chemistry Using Quantitative Structure–Activity Models

J.M. Delieu[1,*], R.W. Horobin[2] and J.K. Duguid[3]

[1]*Bangor University, School of Health Care Sciemces, Archimedes Centre, Wrexham Technology Park, Wrexham, UK;* [2]*Division of Neuroscience & Biomedical Systems, Institute of Biomedical & Life Sciences, Glasgow University, Glasgow, UK;* [3]*Dept of Haematology, Wrexham Maelor Hospital, Wrexham, UK*

Abstract: An investigation of the relationships between physicochemical features of ten antipsychotic drugs and previously reported influence of these drugs on neutrophil maturity was made. A quantitative structure-activity relations (QSAR) approach was adopted, in which several numerical parameters describing physicochemical characteristics of the antipsychotics were estimated. Possible connections between these parameters and neutrophil maturity were explored. Influence of drug physicochemistry on the incidence of agranulocytosis and neutropenia reported in the literature was documented. Overall it was found that drugs with the greatest tendency to induce neutrophil immaturity (chlorpromazine, clozapine and olanzapine) also showed the greatest tendency to cause agranulocytosis and neutropenia. Moreover marked induction of neutrophil immaturity occurred with compounds of moderately amphipathic character, whose amphipathic indices (AI) fell in the range 3–5; higher or lower AI values correlated with less immaturity. Consideration of the QSAR findings suggest that toxicity could be associated with selective uptake into the most fluid intracellular membranes, those of the endoplasmic reticulum and the outer mitochondrial membrane. The AI hazard zone (AI = 3–5) does constitute a predictive tool to assess risk of agranulocytosis and neutropenia arising from antipsychotic and other psychoactive drugs — and not only risk arising from medication but also from experimental or even proposed compounds.

Key Words: Agranulocytosis, antipsychotics, neutropenia, QSAR, chlorpromazine, clozapine, olanzapine, risk prediction.

INTRODUCTION

Antipsychotic drugs have been used for nearly half a century as the first-line treatment for persons diagnosed with schizophrenia and other psychotic disorders. The number of patients so treated is considerable, since the lifetime risk of being diagnosed schizophrenic is 0.7–0.9% [1]. Once diagnosed, patients are maintained on medications indefinitely, resulting in a considerable lifetime risk of side effects [2]. Consequently the number and severity of adverse drug reactions caused by antipsychotic drugs is of concern [3-4]. Agranulocytosis, a potentially fatal although infrequent rapid fall in circulating neutrophil numbers, has a frequency around 1% per annum per patient taking antipsychotic medication [5]. Various mechanisms underlying such haematological toxicity have been discussed, including both immunological processes [6-8] and direct toxicity, sometimes involving metabolic derivatives [9]. Currently there is no way of identifying patients at risk of this serious problem, nor of predicting which chemical structures are likely to be most implicated in this regard [10].

It has recently been found that patients (n = 212) medicated with a variety of typical and atypical antipsychotic drugs had abnormally immature neutrophil populations, despite the total white blood cell and neutrophil counts falling in the normal range [11,12]. For some drugs significant dose-response relationships have been found, and an increase in neutrophil immaturity has also been demonstrated following commencement of such medications. Development of agranulocytosis has been demonstrated in a patient who was previously shown to have an extremely immature neutrophil population.

Existing antipsychotic drugs show marked differences in such haematological toxicity, clozapine being regarded as the most hazardous, and chlorpromazine also being notable for its toxic effects [13]. It was therefore of interest that neutrophil immaturity also varied markedly between drugs, with chlorpromazine and clozapine producing the greatest reductions in maturity.

Given the numbers of patients at risk it seemed of both clinical and scientific interest to investigate possible correlations between the occurrence of such haematological hazards and the chemical structures of the drugs concerned. If such relationships exist, the prediction of risks arising from drugs in clinical use, from experimental drugs, or indeed from proposed drugs may become possible.

As mentioned above, various causes of haematological toxicity have been discussed, and no consensus has yet been reached. There is however a widely applicable semi-empirical tactic for correlating complex biological events with the chemical features of the compounds involved. This is quantitative structure–activity relations (QSAR) modeling, with recent exemplar applications being the analysis of such com-

*Address correspondence to this author at the Bangor University, School of Health Care Sciemces, Archimedes Centre, Wrexham Technology Park, Wrexham, UK; E-mail: hss004@bangor.ac.uk

1573-4064/09 $55.00+.00

plex chemical–biological interactions as responses to anti-inflammatory drugs [14] and effects of toxic compounds on aquatic ecosystems [15]. We therefore investigated the relationships between physicochemical features of antipsychotic drugs and the previously measured neutrophil immaturity; and also with the incidence of reported haematological hazards.

This required estimation of a variety of numerical parameters describing the physicochemical features of antipsychotic drugs, of which brief accounts follow. A measure of the overall size of the aromatic (conjugated) system of a molecule is provided by the conjugated bond number (CBN). Exceptionally large CBN values (> 40), as seen with certain experimental tetraphenylporphine photodynamic therapy drugs, correlate with strong binding to membrane proteins; the well known polyaromatic carcinogen benzpyrene has a CBN value only slightly more than half this, and is therefore membrane permeable. A measure of the hydrophilicity–lipophilicity of a drug is provided by the logarithm of the octanol–water partition coefficient (log *P*). Positive values of which indicate lipophilicity, negative values hydrophilicity. Molecules containing distinct lipophilic and hydrophilic domains are termed amphipathic (synonym: amphiphilic). Their surface properties may be modelled in part using the estimated log *P* value of the lipophilic domain. This parameter is termed the amphipathic index (AI). Significant surface effects are usually only seen when AI > 3.5; non-selective insertion into all membranes occurring when AI > 5; and strong binding to membranes at AI > 8. The actual electric charge (Z) on a molecule depends on the acid or base or strength of the relevant substitutents, here specified using the negative logarithm of the equilibrium constant of the more and less protonated species (the pK_a value).

Relationships were sought between these parameters and neutrophil maturity, both directly and *via* predictions of intracellular localisation. Analogous relationships with reported agranulocytosis and neutropenia were also investigated.

MATERIALS & METHODS

Immaturity of Circulating Neutrophil Populations

The measured index of immaturity used in the studies of patients diagnosed with schizophrenia and treated with various antipsychotic medications, and in healthy non-medicated control populations, was the mean or median of the mean lobe number (MLN) of neutrophil nuclei [11,12]. The MLN was assessed in a standard manner on peripheral blood smears. Such MLN measurements were obtained from a total of 212 patients, treated with ten different antipsychotic drugs. From this experimental dataset the median values of the MLN were obtained, and these are given in Table **1**. Full details are provided in the two papers cited.

Identifying Groups of Psychopharmaceutical Agents from the Literature

We sought to indentify comparible datasets, of two types, which were reported to induce relevant haematological pathology. These were antipsychotic drugs not studied by us, and drugs from additional classes of psychoactive drugs. The procedures adopted were to insert appropriate search strings (e.g. [medication OR drug OR antipsychotic] AND [agranulocytosis OR neutropenia]) into *Entrez PubMed*. Similar procedures were implemented with *OVID*, *PsychINFO* and *Embase*. To avoid cherry-picking the data, a dataset of drugs falling into the category 'antipsychotic drugs not inducing haematological pathology' was generated by using the listing of 70 antipsychotic drugs given in the 'therapeutic category and biological activity index' of the 14th edition of *The Merck Index* [16]. Since many of these will not have been widely used clinically, a further filter was applied. Namely that only those drugs with ≥ 100 hits in the *Entrez PubMed* database were considered further.

Numerical Structure Parameters and how they were Obtained

The amphipathic index (AI), electric charge (Z), logarithm of the octanol-water partition coefficient (log *P*), and size of the total aromatic system (CBN, or conjugated bond number) were obtained by inspection of structural formulae and by calculation. Procedures were usually as previously summarized [17]. Log *P* values were calculated manually using the procedures of Hansch and Leo [18] since software for log *P* computation available to us did not provide values for cationic species. The amphipathic index (AI), a measure of the lipophilicity of the largest lipophilic domain of amphipathic compounds, was obtained using the procedure of Christensen *et al.* [19]. The values of the basicity measure (pK_a) were obtained from the United States National Library of Medicine's online database *ChemIDplus Advanced* and the 14th edition of the *Merck Index* [16].

Relationships of Various Structure Parameters with the MLN values

The mean and median values of the MLNs of the antipsychotic drugs previously studied by us did not differ in their ordinal values, so for simplicity we here use the median values only, as tabulated in Table **1**. The relationships of MLN, in our sample of patients medicated with antipsychotic drugs, to the various structure parameters were explored.

Prediction of Intracellular Localization

Various QSAR models have been published which predict localisation sites of xenobiotics within living cells. In this study the QSAR models used were those for predicting permeant/impermeant behaviour [17]; non-specific accumulation in all biomembranes [17]; and specific accumulation in endoplasmic reticulum [20], lysosomes [21], mitochondria [22], nuclei [23], and the plasma membrane [17]. To make such predictions, the values of structure parameters for individual drugs were compared to the physicochemical specification of compounds accumulating in the various intracellular structures, as provided by the QSAR models. Those compounds falling within the relevant regions of parameter space are then expected to accumulate at the specified sites.

RESULTS

Structure Parameters Estimated and Otherwise Obtained

Parameter values for the antipsychotic drugs previously investigated by us are summarised in Table **1**. The AI and

Table 1. Mean Lobe Numbers (MLNs), Various Physico-Chemical Properties, and Predicted Intracellular Localisations of Antipsychotic Drugs Previously Investigated by us — Ranked in Order of Decreasing Median MLN

Drug Name	Median MLN	AI [1]	CBN [1]	log *P* [1]	Z [1]	pKa	Predicted Intracellular Localisations and Properties [2]
Sulpiride	2.61	0.3	10 10	-4.2 0.4	1+ 0	9.0	L Membrane permeant
Haloperidol	2.53	2.6	16 16	-1.1 4.0	1+ 0	8.7	L Membrane permeant
Flupenthixol	2.5	5.9	17 17 17	-2.4 1.4 5.7	2+ 1+ 0	dibasic	L Membrane permeant ER M Membrane permeant BioM
Thioridazine	2.48	5.1	15 15	0.9 6.0	1+ 0	9.5	Membrane permeant ER M Membrane permeant BioM
Risperidone	2.45	2.7	18 18	-2.1 3.2	1+ 0	basic	L Membrane permeant
Fluophenazine	2.44	5.2	14 14 14	-3.0 0.5 5.1	2+ 1+ 0	3.9 8.1	L Membrane permeant ER M Membrane permeant BioM
Trifluoperazine	2.4	5.3	14 14 14	-2.7 1.1 5.5	2+ 1+ 0	3.8 8.2	L Membrane permeant ER M Membrane permeant BioM
Olanzapine	2.34		18	-4.3	2+		L
		3.5	18 18	0.0 4.2	1+ 0	dibasic	Membrane permeant ER M Membrane permeant
Clozapine	2.34	3.8	18 18 18	-3.9 0.4 4.6	2+ 1+ 0	3.7 7.6	L Membrane permeant ER M Membrane permeant
Chlorpromazine	2.3	4.5	15 15	1.0 5.3	1+ 0	9.2	Membrane permeant ER M Membrane permeant BioM

1 AI = amphipathic index; CBN = conjugated bond number; log P = log of octanol-water partion coefficient ;
Z = electric charge.

2 Uptake sites coded as follows: BioM = generic biomembranes; ER = endoplasmic reticulum;
L = lysosome; M = mitochondrium.

log *P* values for other groups of drugs (antipsychotic and other classes of psychoactive drugs which are reported to induce agranulocytosis or neutropenia; and antipsychotic drugs not inducing such toxic effects) were also estimated, and AI values are displayed in Fig. (**2**).

The relationships of structure parameter values with median MLNs was explored, both by consideration of the data summarized in Table **1**, and for the AI parameter graphically in Fig. (**1**). Note that in this figure, a plot of MLN against AI, the line shown represents a fitted quadratic equation; both coefficients of which were significant at $P < 0.05$.

The prediction of intracellular drug localisation was carried out using QSAR localisation models, and the predictions emerging from these models for the antipsychotic drugs previously investigated by us are listed in Table **1**.

An account of the development of structure parameter criteria for predicting haematological toxicity is described in the Discussion. The final specification of the compounds with potential for haematological toxicity can be expressed as follows:

$$3.5 < AI_{monocation} \text{ or } \log P_{monocation} < 5$$

$$AI_{unionised\ species} \text{ or } \log P_{unionised\ species} < 5$$

$$Z_{ionised\ species} > 0;\ c\ 7 < pK_a < 10;\ CBN < 40$$

(where AI is the amphipathic index, log P the octanol-water partition coefficient, Z the electric charge and CNB the conjugated bond number).

Various datasets for testing the criteria defining the haematological hazard zone of AI values were identified. The estimated AI and log *P* values of certain of these are listed in Table **2**.

The value of the AI structure parameter in particular for delineating a potential 'haematological risk factor zone' is

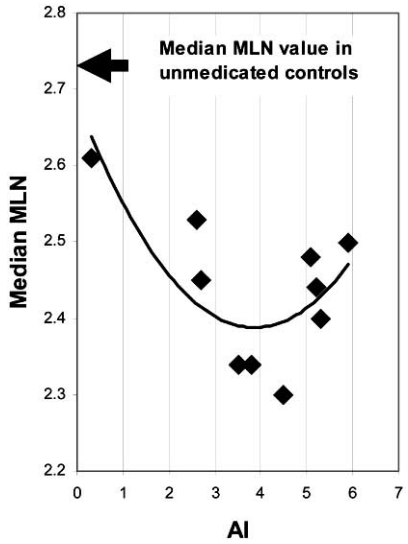

Fig. (1). A plot of median mean lobe number (MLN) against the amphipathic index (AI) for the ten antipsychotic drugs previously investigated by us. The median MLN value for unmedicated, healthy controls is also indicated. The line was fitted using a quadratic function, both of the coefficients having $P < 0.05$.

displayed graphically in Fig. (**2**). This figure aggregates data from compounds noted in both the tables, in addition to extra compounds.

DISCUSSION

Relationship of Structure Parameters with the Median MLN Values

Inspection of the data in Table **1**, where drugs previously studied by us are ranked in order of decreasing median MLN value, failed to reveal obvious linear relationships between the median values of the MLNs in patient groups medicated with the various antipsychotic drugs and any of the structure parameters. However when the median MLN values were plotted against the various structure parameters, a statistically significant U-curve was observed in the MLN–AI diagram, see Fig. (**1**).

This figure provokes the question: why should changes in AI correlate in this way with changes in the maturity of neutrophil polymorphs? Such differences in biological outcome could reflect different patterns of intracellular drug localisation, and this topic is addressed next.

Predicting Intracellular Drug Localisation using QSAR Models

Useful generalisations concerning physicochemical properties of the drugs previously studied by us can be made on the basis of Table **1**. All the drugs are bases which, given the known or probable pK_a values, will comprise both cationic and neutral (free base) species under physiological conditions. The lipophilicities of the mono-cations vary from hydrophilic to lipophilic, whereas those of the neutral species

Table 2. Amphipathic Indices (AI) and log P Values of Centrally Acting Psychoactive Pharmaceutical Agents Described in the Literature as Inducing Agranulocytosis and/or Neutropenia, Together with Ancillary Information

Drug name	AI	log *P* [1]	Therapeutic category	Exemplar description of role in haematological pathology
Amoxapine	3.5	0.3	Antidepressant	Sedlacek, Rudolf, Kaehny [33]
Carbamazepine	4.0	2.9	Mood stabiliser	Sedky, Lippmann [34]
Dipyrone/metamizole	4.2	-0.3	Analgesic	Bonkowsky *et al.* [35];Hamerschlak,Cavalcanti [36]
DMP 406[2]	3.7	-0.8	Experimental antipsychotic	Lorenz *et al.* [37]
Fluperlapine	3.4	-0.4	Antipsychotic	Lai *et al.* [38]
Indalpine	4.0	-0.3	Antidepressant	Healy [39]
Loxapine	4.6	0.8	Anxiolytic	Hehn *et al.* [40]
Mianserin	4.1	-0.1	Antidepressant	van der Klauw *et al.* [41]
Mirtazapine	3.7	-1.3	Antidepressant	Anghelescu *et al.* [42]
Perazine	3.5	0.2	Antipsychotic	Grohmann *et al.* [43]
Prochlorperazine	4.2	0.9	Antipsychotic	McFarland [44]
Promazine	4.3	0.3	Antipsychotic	Holmes, Barg [45]
Quetiapine	3.2	0.7	Antipsychotic	Ruhe *et al.* [46]
Sertraline	5.3	2.1	Antidepressant	Trescoli-Serrano & Smith [47]
Triflupromazine	4.2	1.2	Antipsychotic	Ayd [48]

1 For all but carbamazepine (an amide) and dipyrone (a weak acid) it is the log P value of the cationic species is given here.

2 Agranulocytosis seen in experimental animals (dog).

are without exception lipophilic. Dications when present are hydrophilic. The amphipathic properties of the mono-cations implied by the AI parameter vary from trivial to strong. All the drugs have small aromatic systems.

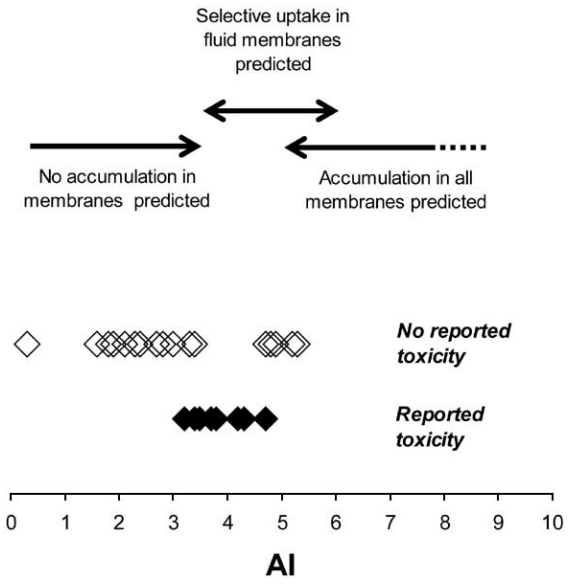

Fig. (2). Antipsychotic drugs reported to give (♦) or not to give (◊) agranulocytosis or neutropenia, plotted on an amphipathic index (AI) diagram. Also plotted are certain membrane-uptake predictions.

Using parameter data and the QSAR models mentioned above, predictions of certain localisations and behaviour have been made, see Table **1**. Since under physiological conditions all the drugs contain lipophilic (non-ionised) species, and because all the drugs have fairly small aromatic systems, all are expected to be membrane permeable. Once inside the cell, accumulation in a variety of structures — biomembranes in general, endoplasmic reticulum, lysosomes, mitochondria — is predicted; whilst selective accumulation in nuclei and the plasma membrane is not expected for any of these compounds. Note that although little has been published concerning intracellular localisation of antipsychotic drugs, available reports are congruent with these predictions. For instance, Zhang *et al.* [24] using secondary ion mass spectroscopy microscopy showed that trifluoperazine did not accumulate in the cell nucleus or plasma membrane, but rather in the cytoplasm.

Predicted intracellular localisations for drugs falling into the three segments of the MLN–AI plot will now be summarised. First consider weakly amphipathic drugs (AI < 3.5), which induce small reductions in neutrophil maturity: haloperidol, risperidone and sulpiride. These are predicted to accumulate only in lysosomes. Second consider drugs of moderate amphipathic character (5 < AI < 3.5), which give rise to appreciably immature neutrophils: chlorpromazine, clozapine and olanzapine. These are predicted to accumulate in endoplasmic reticulum and mitochondria, as well as in lysosomes. Third consider strongly amphipathic drugs (AI > 5) which again result only in small reductions in neutrophil maturity: flupenthixol, fluphenazine, thioridazine and trifluoperazine. This latter group is predicted to accumulate non-selectively in all biomembranes, not merely in those of endoplasmic reticulum, lysosomes and mitochondria. The three segments of the MLN–AI plot therefore correlate with three patterns of expected intracellular accumulation.

Contrast the drugs with the greatest tendency to induce neutrophil immaturity, which are predicted to accumulate in endoplasmic reticulum and mitochondria, with the two groups not inducing appreciable neutrophil immaturity. One of these latter groups is not predicted to accumulate in endoplasmic reticulum or mitochondria, the other could do so, although only in competition with other organelles. This is consistant with the view that the endoplasmic reticulum and mitochondria have particular importance. So it is significant that compounds which accumulate in endoplasmic reticulum are routinely observed to first accumulate in mitochondria [20]. Although these authors were unclear on this point, it is apparent that uptake into these two organelles is favoured because of the high fluidity of their membranes, resulting from the low cholesterol content of endoplasmic reticulum and of the outer mitochondrial membrane [25]. Indeed preferential uptake of lipophilic compounds into endoplasmic reticulum and mitochondria is a well documented phenomenon [26].

Consequently prediction of uptake into endoplasmic reticulum also implies uptake in mitochondria by a mechanism additional to those addressed by the QSAR model for mitochondrial localisation used above. Uptake into these structures may thus be ascribed, at least in part, to a 'most fluid membranes' mechanism. In the light of this conclusion, it is of interest that a number of recent experimental investigations indicate that antipsychotic agents are associated with adverse mitochondrial events [27-30].

Structure Parameter Criteria for Haematological Toxicity

Whilst the QSAR models discussed above concern only intracellular localisation, these findings do have implications for cellular toxicity. For instance the literature considers some antipsychotic drugs to have potentially cell-damaging properties, e.g. formation of oxidative and reactive species [31,32]. Consequently the predicted localisations suggest possible intracellular sites at risk; and the most fluid membranes could be the key structures. Note that the structural features of drugs giving rise to drug accumulation at some particular site may have no relationship to the features which caused cellular damage.

If this analysis is broadly valid, the physicochemical features of drugs constituting potential haematological hazards can be specified. First, such drugs must have access to cellular interiors. This requires both lipophilicity and an absence of trapping in either the lipid or protein domains of the plasma membrane or endosomes. This may be parameterised [17] as follows:

$$CBN < 40;\ 0 < \log P_{\text{unionised species}} < 8$$

(abbreviations are those defined in the Materials and methods section)

Within the cell, potentially hazardous drugs must accumulate in mitochondria and endoplasmic reticulum, which

phenomena have previously [22, 20] been defined. In the present context, and assuming entry through the plasma membrane, these can be expressed as follows:

$$0 < \log P_{\text{monocation}} < 5$$

or

$$3.5 < \log P_{\text{monocation}} < 6;\ 3.5 < \text{AI}_{\text{monocation}} < 6$$

for mitochondria and endoplasmic reticulum respectively.

Such characteristics preclude selective accumulation within lysosomes, nuclei or the plasma membrane [21, 23, 17]. Substantial and competitive non-selective accumulation in biomembranes — e.g. of Golgi complex, lysosomes, mitochondria, plasma membrane and so on — will not occur so long as the amphipathic and lipophilic character of the species present is not excessive. This requirement may be expressed so:

$$\text{AI}_{\text{unionised species}} \text{ or } \log P_{\text{unionised species}} < 5$$

The above account has assumed that the compounds are reasonably strong bases, as indeed is so for these antipsychotic drugs. Consequently two further parameters need specifying:

$$Z_{\text{ionised species}} > 0;\ \text{c } 7 < \text{p}K_a < 10$$

These specifications may be combined, to acknowledge the significance of accumulation in endoplasmic and outer mitochondrial membranes, and of the competitive effect of non-selective uptake into other membranes. An overall statement of possible haematological hazard criteria can be proposed, as follows:

$$3.5 < \text{AI}_{\text{monocation}} \text{ or } \log P_{\text{monocation}} < 5$$

$$\text{AI}_{\text{unionised species}} \text{ or } \log P_{\text{unionised species}} < 5$$

$$Z_{\text{ionised species}} > 0;\ \text{c } 7 < \text{p}K_a < 10;\ \text{CBN} < 40$$

All the typical and atypical antipsychotic drugs previously investigated by us meet the criteria for $\text{AI}_{\text{unionised species}}$, $\log P_{\text{unionised species}}$, $Z_{\text{ionised species}}$, and $\text{p}K_a$ values. Consequently it is expected that the most risky antipsychotic drugs, which are all of moderate amphipathic character, are identifiable by the AI value of their monocations falling into a limited range, namely:

$$3.5 < \text{AI}_{\text{monocation}} \text{ or } \log P_{\text{monocation}} < 5.$$

Given these criteria, various questions arise. How widely applicable are the criteria? Do antipsychotics, and indeed other psychoactive drugs, identified in the literature as inducing agranulocytosis or neutropenia typically have AI values in or near the specified range? Moreover, is the assumption that neutrophil immaturity predicts risk from such haematological pathology valid?

Relationship of Neutrophil Maturity, the AI Effect and Haematological Pathology

The previous section sought to correlate drug chemistry with induction of neutrophil immaturity. However, an immature neutrophil population is not in itself pathological, only an indication of some demand on or stress of the neutrophil system — and perhaps of a predictor of future pathology. Here evidence is discussed which supports a connection between neutrophil immaturity and haematological pathology, and in addition the value of the AI parameter for identifying potentially risky drugs.

As summarised in Table **2** of Delieu *et al.* [12], of the ten antipsychotic drugs investigated by us, three in particular (chlorpromazine, clozapine and olanzapine) are associated with literature reports of agranulocytosis or neutropenia. These drugs are those with the lowest median MLN values, and their AI values fall in the range 3.5–4.5. An effort was then made to check on these preliminary conclusions, and to widen the field of view. A literature search (details as described in Materials and Methods above) identified an additional seven antipsychotic drugs, listed in Table **2**, which were reported to give rise to agranulocytosis or neutropenia. Some drugs in this dataset were experimental, so multiple reports of pathology were not required for inclusion. The AI values for this group of drugs fell in the range 3.2–4.3. The distribution pattern of the summed antipsychotic dataset is shown in Fig. (**2**).

Using analogous criteria eight psychoactive drugs of other therapeutic categories, listed in Table **2**, were identified which were reported as inducing haematological pathology. For these compounds — mostly bases with membrane-permeant neutral species, although one was a lipophilic and amphipathic amide (carbamazepine) and one was a weak acid with a membrane permeant free acid (dipyrone) — the AI values fell in the range 3.5–5.3.

Finally a fourth dataset of 23 psychoactive drugs was generated, comprising antipsychotic agents without reported haematological pathology. The procedure used to avoid cherry-picking this dataset is described in the Methods section. These were permeant bases, whose AI values fell in the range 0.3–5.3. These compounds are plotted in Fig. (**2**), inspection of which shows their distribution. Although covering a range of nearly four AI units, the only gap — from 3.4–4.7 — overlapped the AI range of those antipsychotic drugs which induce haematological pathology.

In the light of these observations the following statements can be made:

- Drugs with the greatest tendencies to induce neutrophil immaturity also show the greatest tendencies to cause agranulocytosis and neutropenia using the criteria specified above.
- Induction of neutrophil immaturity, and risk of agranulocytosis and neutropenia, is restricted to compounds whose AI values fall in the range 3–5, or immediately adjacent to it.
- The significance of this range of AI values is reinforced by the complete group of datasets.
- The dataset of psychoactive drugs additional to the antipsychotics suggests that toxicity is due to some direct intracellular event(s), not receptor mediated or illness-related effects.
- The parallel between the AI 3–5 risk zone and the AI values associated with selective uptake into the most

fluid intracellular membranes — namely those of the endoplasmic reticulum and the outer mitochondrial membrane — on the one hand, and the onset of non-selective uptake into biomembranes, are congruent with a specific intracellular location for the toxic effect(s). Fig. (**2**) provides a graphic summary of these phenomena. The limited correspondence seen in the figure is perhaps due to the, biologically unrealistic, dichotomous character of the QSAR models.

Prediction of Haematological Pathology and Drug Toxicity

First consider prediction of the haematological risks for individual patients. These risks cannot be predicted at the present time, however the approach described here might assist in such predictions, by measuring some function of the MLN of the patient's neutrophil population. However what absolute MLN value, or change in MLN value for the patient, would be predictive of agranulocytosis or neutropenia would need a prospective study; and of course other factors such as duration of medication would also have to be taken in consideration. Since the incidence of agranulocytosis is low, this would require following up a large number of patients being treated with antipsychotic medication over several years. To make the necessary, repeated, haematological assessments feasible this would also call for replacement of the manual assessment of MLN, as used in our two prior studies, with suitable output from an automated cell counter, standardised against a manual MLN count. This would itself require methodological and instrumental development work. So the question which must be asked is: would such a program be worth while? Given the number of patients at ongoing risk, and the costs of treatment, and the possibility of choosing less toxic drugs on a rational basis — we suggest the answer is "yes".

Second, consider the prediction of particularly risky antipsychotics. The approach outlined here can already assist in this, *via* the concept of the AI 3–5 hazard zone. Chlorpromazine and clozapine of course are already known to be relatively risky, and do indeed fall in that range. To verify or refute such predictions, a prospective study would again be required. This would have the additional problem of identifying small groups of patients treated by monotherapy with certain low-sales or recently introduced drugs. However it would be possible for psychiatrists with a conservative stance to prescribing to note that certain antipsychotics, that fall into the haematological hazard zone, might be avoided when treating patients over whom there was haematological concern.

Finally consider prediction of potential risks associated with experimental, or proposed, antipsychotic drugs. In principle this can be achieved in exactly the same way as in the previous case. Moreover, proposed chemical structures could be tailored to have AI values outside the risk zone, by adding or removing lipophilic and hydrophilic substituents.

ACKNOWLEDGEMENTS

We acknowledge Dr Peter R Hudson, Principal Biochemist at Maelor Hospital, Wrexham, for statistical advice; and Prof W Martin at the Division of Neuroscience & Biomedical Systems, IBLS, University of Glasgow for providing facilities to one of us (RWH).

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Received:, 15 July, 2008 Revised:, 21 October, 2008 Accepted:, 11 November, 2008

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