THERAPEUTIC MONITORING OF ANTIPSYCHOTIC DRUGS

Schizophrenia affects some 1% of adults during their lifetime and can be a very serious illness - about 10% of patients die an unnatural death, usually suicide. In the UK some 210,000 people (worldwide of the order of 50 million) suffer from the disease. Families, friends, and others are of course affected indirectly. Today the estimated cost of treating schizophrenia is 2.5% of total healthcare costs in Western countries – for the NHS 2008-9 this amounts to some £2.4 billion; there are additional contributions from the private sector and other agencies such as the Home Office.

Schizophrenia is a chronic disorder, typically arising in early adulthood and progressing throughout the rest of the individual's life. It is slightly more common in males than females, but usually occurs earlier and is more serious in males. Symptoms are protean, but are generally characterised by three groups:

- ***'Positive': delusions, hallucinations, disorganised behaviour, impaired communication.***
- ***'Negative': social withdrawal, poverty of thought and speech, blunted affect, lack of drive.***
- ***Cognitive dysfunction: affecting insight, memory, reasoning, etc.***

Chromatography Focus

Nowadays the mainstay of treatment is drug therapy. Chlorpromazine, haloperidol and other 'first generation' drugs are gradually being superseded by 'second generation' or 'atypical' antipsychotics that generally have fewer unpleasant side-effects than the older drugs.

The most effective of these second generation drugs is clozapine. There are a number of reports that demonstrate relationships between plasma antipsychotic concentrations and in vivo binding to receptors in different regions of the brain. However, only in the case of plasma clozapine is there evidence that there is a generally-applicable 'target range' associated with successful therapy making it an ideal candidate for therapeutic drug monitoring (TDM) (Flanagan, 2006; Hiemke, 2008).

THE ADVENT OF CLOZAPINE

Clozapine is used only in patients unresponsive to, or intolerant of, other antipsychotics. Clozapine seems to work in some 30-50% patients who do not respond fully to other medicines, and is better tolerated by others who could not take older drugs.

Because of the risk of agranulocytosis (some 2% of patients given the drug develop neutropenia), white cell counts are monitored regularly throughout treatment (Flanagan & Dunk, 2008). Prompt withdrawal of clozapine once impending neutropenia/agranulocytosis is detected usually results in the white cell count returning to normal.

The occurrence of neutropenia/agranulocytosis is unpredictable, although there is a dose-related component. Clozapine also has a number of dose-related adverse effects, most notably sedation and convulsions, although patients do become tolerant to these effects to an extent on chronic dosage (Yusufi et al., 2007).

There are further complications in using clozapine. Firstly, the full therapeutic benefit may take weeks or months to become apparent. Secondly, there is an approximately 50-fold between-patient variation in dose requirement due to genetic differences in the ability to metabolise the drug. Thirdly, dose requirement is markedly (±50 % on average) affected by smoking habit, a problem exacerbated by moves to impose a smoke-free NHS (Rostami-Hodjegan et al., 2004). Finally, clozapine may cause constipation, which may impair absorption of the drug.

As noted above, some adverse effects of clozapine are associated with higher doses/higher plasma concentrations. On the other hand, several studies have suggested that a 'steady-state' plasma 'total' (i.e. free + protein bound) clozapine concentration of at least 0.35mg L^{-1} is associated with a good response, whilst there is thought to be an increased risk of seizures at clozapine doses above 600 mg d^{-1}, doses which are on average associated with plasma clozapine concentrations above 0.6 mg L^{-1} or so. However, there is wide inter-individual variation reflecting the wide genetic variation in the ability to metabolise clozapine as discussed above *(Figure 1)*.

A further problem with using clozapine is that it has to be given orally, sometimes raising questions of adherence with the prescribed dose. In addition to assessing adherence, monitoring the plasma concentration of clozapine and of its *N*-desmethyl metabolite norclozapine in a 'trough' (i.e. pre-dose) sample maximises the chance of maintaining a good response to the drug whilst guarding against overdosage. Measuring the plasma clozapine concentration can confirm that clozapine has received an adequate trial and that supplemental strategies, for example augmentation with other drugs such as sulpiride, amisulpride, or lamotrigine, are indicated in partial responders.

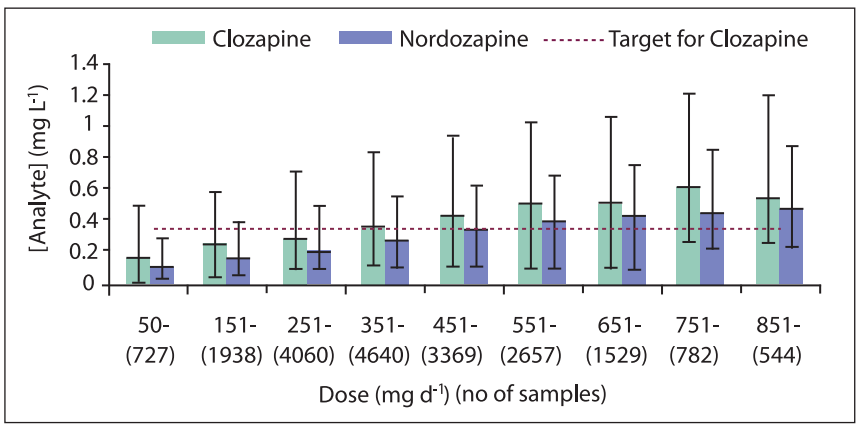

Figure 1. Plasma Clozapine/Norclozapine vs. Dose (median, 10th & 90th percentiles; n = 20,156).

Author Details:

Dr Bob Flanagan BSc PhD ERT RFP CChem FRSC FRCPath is Consultant Clinical Scientist at the Toxicology Unit, Department of Clinical Biochemistry, King's College Hospital NHS Foundation Trust, Denmark Hill, London SE5 9RS

HPLC OF CLOZAPINE AND NORCLOZAPINE

The methodology used to measure clozapine and norclozapine is based on simple one-step microextraction into methyl *tert*-butyl ether (MTBE) (Flanagan et al., 2006). Nortriptyline is used as the internal standard (IS). Use of strong cation-exchange modified silica packings such as Waters Spherisorb S5SCX together with methanol containing an ionic modifier (35 mmol L^{-1} ammonium perchlorate, pH* 6.9) and UV detection (240 nm) allows the solvent extracts to be analysed directly.

These systems have several further advantages over conventional reverse-phase systems. Use of the relatively non-viscous methanol as eluent solvent gives good efficiencies and long column life. A non-eluting injection solvent such as MTBE (no ionic strength) permits relatively large volume sample injection with no loss of efficiency.

The chromatographic system has a high degree of selectivity since only protonated basic drugs are retained. Ammonium perchlorate is a valuable ionic modifier since it is adequately soluble in methanol and has no UV absorption down to ca. 205 nm.

A disadvantage to the use of ammonium perchlorate is that it is seemingly incompatible with MS detection. However, substitution of methanolic ammonium acetate of equivalent ionic strength and pH* as eluent circumvents this problem if MS detection is needed in other applications.

Used with standard HPLC equipment, Waters Spherisorb S5SCX in a conventional 125 x 4.6 mm i.d. column (eluent flow-rate 1.2 mL min^{-1}) gives a 25 min analysis time *(Figure 2)*.

However, use of a 100 x 2.1 mm i.d. column packed with this same material in a modern system designed to cope with the higher column efficiencies now available (Jasco X-LC) offers advantages of miniaturisation, i.e. lower flow-rates hence reduced solvent consumption (a flow-rate of 0.5 mL min^{-1} is equivalent to 3.5 mL min^{-1} on a 125 x 4.6 mm column), increased efficiency and sensitivity hence reduced sample size, and decreased analysis time.

The X-LC has minimal extra-column volume (< 10 µL) achieved using narrow-bore tubing for all important connections together with a 4 µL volume detector flow-cell and a detector sampling rate of up to 100 Hz. The column temperature is controlled in a Peltier effect oven giving much improved retention time reproducibility.

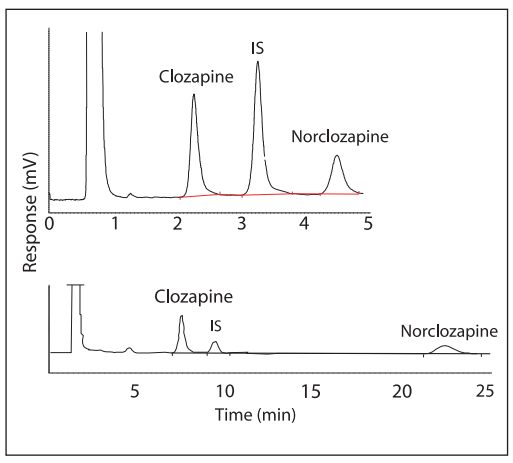

Figure 2. Comparison of 'fast' (100 x 2.1 mm i.d. column, eluent flow-rate 0.5 mL min^{-1}) and conventional (125 x 4.6 mm i.d., eluent flow-rate 1.2 mL min^{-1}) LC in the analysis of clozapine and norclozapine (eluent 35 mmol L^{-1} ammonium perchlorate in methanol, pH 6.9).*

Using this 'fast LC' approach, it has been possible to reduce the sample volume (from 200 to 100 µL) and the extraction solvent (from 200 to 125 µL) requirements and the extract injection volume (to 50 µL) giving lower reagent and calibrator costs (*Figure 3*).

The much reduced analysis time (from about 25 to 5 min) in turn gives increased sample capacity and a much improved turn-around time with no loss in sensitivity/selectivity.

As to future developments, the advent of smaller aps (average particle size) packings of course offers further advantages of increased efficiency hence increased throughput, and research in this area is underway.

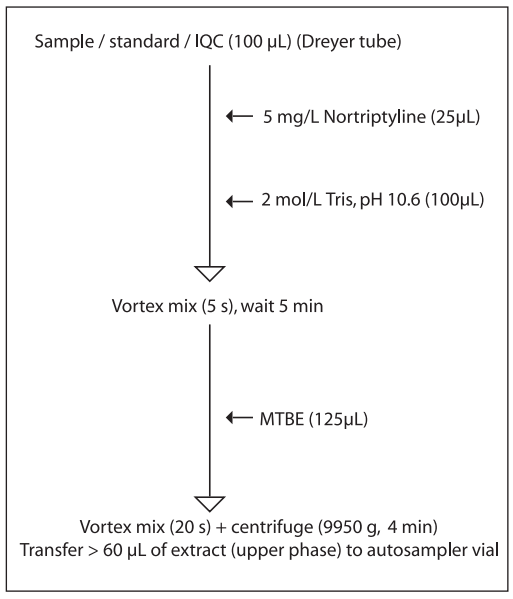

Figure 3. Sample preparation flow-diagram: plasma clozapine assay.

KingsPath PLASMA ANTIPSYCHOTIC ASSAY SERVICE

The KingsPath Toxicology Unit opened in April 2007 with the transfer of the drugs of abuse screening service from the Bethlem Hospital (South London and Maudsley NHS Foundation Trust) to the Department of Clinical Biochemistry at King's College Hospital. KingsPath now offers a clozapine and norclozapine assay service using 0.5 mL plasma with a turnround of 2 working days once the sample is received. Sample collection/mailing kits are available from the Clozaril Patient Monitoring Service (CPMS) or Denzapine Monitoring Service (DMS) for registered patients.

Importantly, the results are available immediately on-line once authorised via NHS net (see: www.kingspath.co.uk for a demonstration of the free KingsPath Results-on–Line service). Urgent results can be faxed or telephoned. Written results are provided by post. Some non-NHS net users can access the Results-on–Line service by special arrangement, other non-NHS users can make arrangement for regular faxing of results. One further aspect of Results-on-Line is that previous results recorded on the system are always available, a valuable facility as regards long-term problems such as assessing adherence to antipsychotic medication.

Some further antipsychotic assays that are available via KingsPath are listed in *Table 1*. The service is similar to that offered for clozapine/norclozapine. LC-MS is used for some analytes. Clinical advice as to the interpretation of analytical results is available in all cases.

KingsPath TOXICOLOGY

KingsPath hosts a Super-regional Assay Service Metals/Trace Elements Laboratory within the Department of Clinical Biochemistry. The advent of inductively coupled plasma-mass spectrometry (ICP-MS) has extended the range of assays available and enhanced reliability.

As to other TDM analytes, assays are being developed and validated for newer anticonvulsant and also for some anti-retroviral drugs. The drugs of abuse screening service is also being enhanced with LC-MS capability, including the ability to confirm the presence of buprenorphine and norbuprenorphine.

Staff training is also an important aspect of the work of the Unit. A modular MSc in Analytical Toxicology hosted by Queen Mary University of London was offered for the first time in October 2007 and is to be offered in subsequent years (www.whri.qmul.ac.uk/courses).

Here at King's, the knowledge gained from studying for the MSc is supplemented by in-house training in different sections of the Department providing a route to State Registration as a Clinical Scientist in Clinical Biochemistry, subspecialty Analytical Toxicology.

REFERENCES

Flanagan RJ. TDM of antipsychotics. CPD Bull Clin Biochem 2006; 7: 3-18.

Flanagan RJ, Dunk L. Haematological toxicity of drugs used in psychiatry. Hum Psychopharmacol 2008; 23(S1): S27-S41.

Flanagan RJ, Morgan P, Spencer EP, Whelpton R. Micro-extraction techniques in analytical toxicology: short review. Biomed Chromatogr 2006; 20: 530-8.

Hiemke C. Clinical utility of drug measurement and pharmacokinetics - therapeutic drug monitoring in psychiatry. Eur J Clin Pharmacol 2008; 64: 159-66.

Rostami-Hodjegan A, Amin AM, Spencer EP, Lennard MS, Tucker GT, Flanagan RJ. Influence of dose, cigarette smoking, age, sex and metabolic activity on plasma clozapine concentrations. J Clin Psychopharmacol 2004; 24: 70-78

Yusufi B, Mukherjee S, R Flanagan R, Paton C, Dunn G, Page E, Barnes TR. Prevalence and nature of side effects during clozapine maintenance treatment and the relationship with clozapine dose and plasma concentration. Int Clin Psychopharmacol 2007; 22: 238-43

Table 1. Some second-generation antipsychotics and reference ranges

	Plasma metabolites	**Suggested Plasma reference range** (µg L^{-1}, pre-dose sample, 5 mL EDTA blood)
Amisulpride	None	100-400
Aripiprazole	Dehydro	Aripiprazole 100-500 Dehydroaripiprazole 40-200
Olanzapine	None	20-40 (12 hour post-dose sample)
Quetiapine	S-oxide, S-oxide carboxylate (both inactive), 7-hydroxy, 7-hydroxy-*N*-desalkyl	Quetiapine 50-200
Risperidone	9-Hydroxy	Risperidone 4-8 (oral dosage) 9-Hydroxyrisperidone 10-25 (oral dosage)
Sulpiride	None	300-500

Dr Bob Flanagan is Director of the Toxicology Unit, King's College Hospital, London. He has worked in analytical/clinical toxicology for 37 years, and has published over 180 papers and four books. Dr Flanagan helped identify volatile substance abuse ('glue sniffing') as an emerging problem in the UK in the early 1980s, and has worked on analytical and forensic aspects of this and many other problems. More recently he has worked on the analysis of psychoactive compounds such as clozapine and olanzapine in plasma and on the clinical interpretation of the results.

Dr Flanagan has recently co-authored a book entitled Fundamentals of Analytical Toxicology, which can be purchased from the ILM Bookstore, **www.ilmbookstore.com**