DEVELOPMENT OF POLYCLONAL ANTIBODIES
FOR THE DETECTION OF ANTIDEPRESSANTS

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Introduction

Antidepressants pharmaceuticals, including escitalopram, sertraline and fluoxetine, are increasingly being prescribed in developed
countries, and have been linked with suicide cases. (1) Escitalopram (Lexapro) is a selective serotonin re-uptake inhibitor (SSRI). (2) Oral
doses of escitalopram (10-20 mg) are normally orally administered once daily, with plasma levels of 3-31 ng/ml and a plasma half-life
average of 22 hours. (3) Escitalopram is metabolised to norceitalopram by demethylation. Sertraline (Lustral, Zoloft), is a
raphaphenazine derivative, is also an SSRI, with oral and daily adult doses ranging from 50-200 mg. (4) Sertraline undergoes N-demethylation to norsertraline, which accumulates in plasma due to slow elimination (62-104 hours). (5) Toxic serum levels of sertraline range from 453-2930 ng/ml. (6)
Fluoxetine (Protest, Prozac) is a phenylpropylamine derivative, with the blockage of serotonin uptake as the proposed mechanism
of action. (7) Fluoxetine is readily absorbed after oral administration (up to 80 mg per day), and its primary active metabolite is
norfluoxetine. (8) Fatal levels of fluoxetine and norfluoxetine in blood are 1300-4600 ng/ml and 900-3000 ng/ml respectivley. (9)

To monitor the use or misuse of antidepressants the availability of effective immunoassays is relevant. The development of antibodies
for the detection of these compounds is of value to generate such immunoassays. This has applications in therapeutic, forensic and
otoxicological settings.

We report the development of four polyclonal antibodies for the detection of different antidepressants.

Methodology

Derivatised escitalopram through N and para positions, derivatised fluoxetine through the N position and sertraline were conjugated to bovine
dryoglobin (BTG) as carrier to generate 4 immunogens. They were then administered to adult sheep on a monthly basis to provide target-
specific polyclonal antiserum. IgG was extracted from the antisera and evaluated via competitive immunoassay.

The purified antibodies were immobilised on a biosensor platform (from n to nmm), which is also the vessel for the immunoassays. The sera-
automated analyser Evidence Investiger (EVI602, Randox Laboratories Ltd., Crumlin, Northern Ireland) was used.

Assay evaluation parameters:

Seroreactivity: Employing a competitive immunoassay format, non-recitalopram, sertraline and fluoxetine calibration curves were generated over 9
concentrations. IBCV values were calculated, where B is the signal measured for x ng/ml of analyte and BO is the signal measured in the absence
of analyte. The IBCV was calculated by taking 50% of signal from the zero calibrator and reading the value from the x-axis (standard concentration
in ng/ml) in the calibration curve.

Precision: intra-assay precision was determined from the results of 6 replicates at different concentration levels within the same run. Results were
expressed as % CV.

Specificity: specificity, expressed as % cross-reactivity (% CR), was calculated as follows;

\[ \text{% CR} = \left( \frac{\text{IC50 (analyte)}}{\text{IC50 (cross-reactant)}} \right) \times 100 \]

Results

Initial evaluation results are reported.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escitalopram</td>
<td>100</td>
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<tr>
<td>Norecitalopram</td>
<td>13.14</td>
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</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>110</td>
</tr>
<tr>
<td>Norfluoxetine</td>
<td>&gt;80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertraline</td>
<td>100</td>
</tr>
<tr>
<td>Racemic norsertraline</td>
<td>149.9</td>
</tr>
<tr>
<td>Norsertraline</td>
<td>93.5</td>
</tr>
</tbody>
</table>

Conclusions

The initial data indicate that the four polyclonal antibodies developed are suitable for the development of efficient
immunoassays for the determination of different antidepressants and metabolites in biological samples. This is of value for
applications in therapeutic, forensic and toxicological settings.

References:

5. Fluoxetine: Clarke’s Analysis of Drugs and Poisons. http://www.medicinescomplete.com