DEVELOPMENT OF A GENERIC MONOCLONAL ANTIBODY AGAINST TRICYCLIC ANTIDEPRESSANTS

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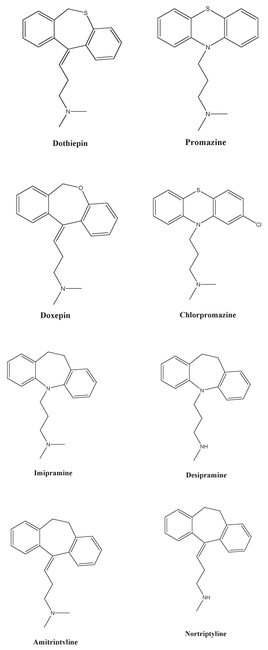
INTRODUCTION

Tricyclic antidepressants (TCAs) are indicated for the treatment of clinical depression, neuropathic pain, nocturnal enuresis and attention deficit hyperactivity disorder (ADHD). Whilst therapeutic drug monitoring (TDM) of TCAs is well established in the treatment of depression, TCA overdose remains the most common cause of death from prescription drugs. [1,2]

We report the development of a monoclonal antibody presenting a broad specificity profile, which will be of value in developing more effective immunoassays for both TDM and toxicological applications.

Chemical structures of some TCAs



METHODOLOGY

Evaluation of the development of the generic monoclonal antibody against TCAs

Selection of fusion wells was carried out by ELISA binding assay using an HRP-labelled tracer blend.
Selection of colonies and further antibody evaluation was performed by competitive ELISA immunoassays in 96-well microtiter plates. Competition between free analyte and horseradish peroxidase (HRP) labelled conjugate for antibody binding sites after incubation of 1 hour at 37°C was measured by reading the absorbance at 450 nm. The absorbance was inversely proportional to the concentration of the analyte.

Assay evaluation parameters:

The IC50 for each analyte was calculated by taking 50% of the optical density (OD) from the zero calibrator and reading this OD value from the x-axis (concentration in ng/ml) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.

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

%CR = [IC50 (Dothiepin)/IC50 (Cross-reactant)] x 100

RESULTS

Selection of 60 fusion wells
(from 672)

OD > 2.0 after reaction with HRP labelled tracer blend (ELISA binding assay)

08/021/NT (PR)

Analysis of supernatants

Supernatants were tested via competitive ELISA to analyse signal displacement (OD 450nm) after separate addition of three different blends of free TCAs (32 ng/ml)and HRP labelled tracer blend (incubation time 1 hour at 37°C).

TCAs blend 1:
Dothiepin Amitriptyline HCl
Doxepin HCl
Nordoxepin HCl
Cyclobenzaprine
TCAs blend 2:
Norchlorpromazine
Imipramine HCl
Chlorpromazine HCl
Desipramine
TCAs blend 3:
Nortriptyline
Protriptyline
Lofepramine

Supernatant NT1.3C11 selected

Selected supernatant	Free TCAs blend 1 (32 ng/ml)	Free TCAs blend 2 (32 ng/ml)	Free TCAs blend 3 (32 ng/ml)
	Signal displacement B/B0		
NT1.3C11	0.17	0.28	0.33

08/023, 099/NT (PR)

NT1.3C11 cells were cloned.

Colonies tested via competitive ELISA to analyse signal displacement (OD 450nm) after separate addition of three different blends of free TCAs and HRP labelled tracer blend (incubation time 1 hour at 37°C).

	Selected colonies NT1.3C11,C11,A5		
	Free TCAs blend 1	Free TCAs blend 2	Free TCAs blend 3
ng/ml	B/B0	B/B0	B/B0
100	0.31	0.27	0.28
32	0.53	0.47	0.47
10	0.76	0.69	0.69
0	1.00	1.00	1.00

08/023, 099/NT (PR)

Colonies NT1. 3C11. C11. A5 selected and re-cloned.

Evaluation of the purified generic monoclonal antibody.

IC50 and specificity

TCA	IC50 ng/ml	Specificity (% cross-reactivity)
Dothiepin	**2.93**	**100.00**
Nortriptyline	2.3	127.39
Protriptyline	1.16	252.59
Lofepramine	1.96	149.49
Doxepin HCl	2.75	106.55
Cyclobenzaprine	1.31	223.66
Trimipramine	1.28	228.91
Imipramine HCl	1.59	184.28
Chlorpromazine HCl	6.51	45.01
Amitriptyline HCl	0.9	325.56
Desipramine	1.08	271.30
Nordoxepin HCl	6.82	42.96
Norclomipramine	4.04	72.52
Promazine	1.24	236.29
Norchlorpromazine	2.58	113.57

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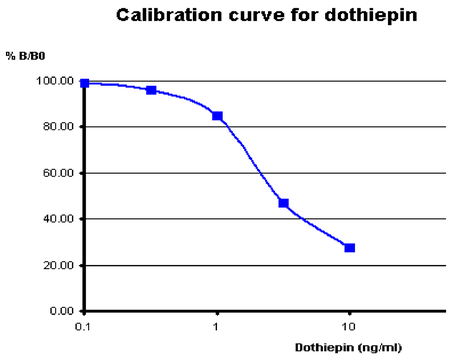

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Comparison with other systems

Developed generic monoclonal antibody		
Total TCAs tested	TCAs cross-reactants	Specificity %Cross-reactivity
15	12	≥100
	1	>70
	2	>40

Sensitivity:
≤ 1.0 ng/ml (relative to dothiepin)
≤ 0.35 ng/ml (relative to imipramine)

Commercial system 1			Commercial system 5		
Total TCAs tested	TCAs cross-reactants	%Cross-reactivity	Total TCAs tested	TCAs cross-reactants	%Cross-reactivity
15	1	100	14	4	>100
Sensitivity	3	20	Sensitivity	7	>50
20ng/ml	11	<10	10ng/ml	3	10

Commercial system 2			Commercial system 6		
Total TCAs tested	TCAs cross-reactants	%Cross-reactivity	Total TCAs tested	TCAs cross-reactants	%Cross-reactivity
11	3	≥100	14	6	≥100
Sensitivity	1	>90	Sensitivity	5	≥40
10ng/ml	7	≤12	75ng/ml	3	<10

Commercial system 3			Commercial system 7		
Total TCAs tested	TCAs cross-reactants	%Cross-reactivity	Total TCAs tested	TCAs cross-reactants	%Cross-reactivity
14	7	>100	10	3	100
Sensitivity NS	3	>60	Sensitivity	1	>50
	4	<30	2ng/ml	6	≤25

Commercial system 4			Commercial system 8		
Total TCAs tested	TCAs cross-reactants	%Cross-reactivity	Total TCAs tested	TCAs cross-reactants	%Cross-reactivity
14	2	>100	11	1	100
Sensitivity NS	8	>90	Sensitivity	5	>50
	4	≤20	20ng/ml	5	<45

* NS=not stated

CONCLUSIONS

- The developed generic monoclonal antibody exhibits **high sensitivity and specificity for a wide range of TCAs and their metabolites.**
- This represents **a significant improvement** for use in developing **more effective immunoassays** applicable to TDM and toxicology fields.

REFERENCES

1. Haddad, L.M. Managing tricyclic antidepressant overdose. *Am. Fam. Physician.* 1992, **46(1)**: 153-159.
2. Pimentel. L. and Trommer, L. Cyclic antidepressant overdoses. A review. *Emerg. Med. Clin. North Am.* 1994, **12(2)**: 533-547.

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