

Chapter 14

The Radioimmunoassay of Nortriptylene and Other Tricyclic Antidepressants

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INTRODUCTION

The tricyclic antidepressant group of drugs are amongst the most widely used medicaments in current clinical practice. They have proved to be extremely useful in the treatment of many different types of depression. There are, however, greater than ten-fold differences between different individual subjects in the rate at which they can degrade the various members of the group into biologically inert metabolites. There is also good evidence that the therapeutic effectiveness of this class of drug is more closely related to their so-called 'steady state' plasma concentrations than to the dose administered. It is clearly important, therefore, to be able to measure plasma drug concentrations simply and rapidly. Methods currently available include fluorimetry, thin layer chromatography (TLC), gas liquid chromatography (GLC), high pressure liquid chromatography (HPLC) and isotope derivatization, but are generally too insensitive, non-specific or slow to be useful clinically.

Radio- and enzyme-immunoassays capable of measuring a number of different tricyclic antidepressants in blood and other fluids have been developed in our laboratory based on an antiserum raised in a sheep⁴. Only the radioimmunoassay will be described here.

ANTISERUM PRODUCTION

The immunogen was prepared by conjugating *N*-4-aminobutyl-nortriptyline to bovine serum albumin (BSA). *N*-4-aminobutyl-nortriptyline was made by refluxing nortriptyline-HCl and *N*-4-(bromobutyl) phthalimide together in dry ethanol for 20 h. The amine was isolated and conjugated with BSA in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide by mixing overnight at room temperature. The nortriptyline-BSA conjugate was purified by dialysis against distilled water and lyophilized under vacuum at 0°C. It was possible, by use of ¹⁴C-nortriptyline tracer carried through the whole procedure, to calculate that only 2-3 nortriptyline residues were coupled to each BSA molecule.

Five milligrams of conjugate and 3 mg BCG vaccine were dissolved in 1 ml sterile saline and emulsified with 2 ml Marcol 52 adjuvant and injected into two sheep at six sites intramuscularly. Pertussis vaccine was injected intradermally at the same time. Booster injections were given at monthly intervals for three months and irregularly thereafter (Fig. 1). Serum collected from one of the sheep (S259) eventually produced an antiserum capable of being used at an initial dilution of 1:1000.

LABEL

The first label available to us was a low specific activity (¹⁴C-*N*-methylnortriptyline (2.13 mCi mmol⁻¹)) generously donated by the Lilly Research Centre, Windlesham, UK.

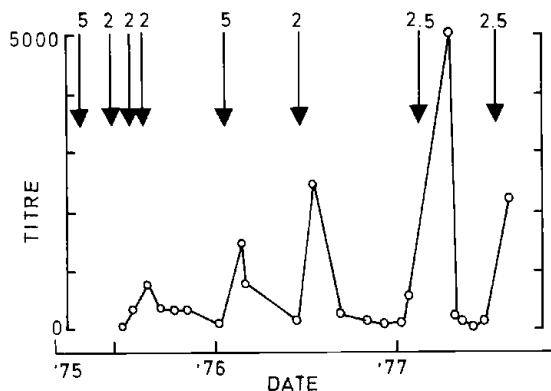


Fig. 1 Time course and immunisation schedule adopted in S259. Arrows indicate timing of booster injections and amount of conjugate (mg).

Although too insensitive to use as a clinical assay, it was possible, using this label, to construct a standard curve and characterize the cross-reactivity of the antiserum. This revealed an extremely high group specificity for tricyclic antidepressants. Within the group, however, the antiserum was capable of binding amitriptyline and imipramine with an avidity about 50% greater and about 20% less than nortriptyline respectively. The antiserum bound several other tricyclic antidepressant drugs markedly less well.

Subsequently, a high specific ^3H -imipramine label (23 mCi mmol^{-1}) became available commercially from Radiochemical Centre, Amersham, and has been used in most of our subsequent work except when comparisons were made with assays set up using a high specific activity ^3H -amitriptyline label generously donated by Lundbeck and Co., Copenhagen.

Using antiserum obtained from S259 and the ^3H -imipramine label, a radioimmunoassay has been set up which is capable of being used on a daily basis for the measurement of tricyclic antidepressants in unextracted plasma from patients treated with them. Results can be made available with a between-batch coefficient of variation of $< \pm 10\%$ within two hours of receipt of the specimen in the laboratory. The assay is sensitive to 1 ng nortriptyline per ml plasma. Up to 50 specimens can be handled in a single run using an automatic scintillation counter and semi-automated reagent dispensers. Detailed assay conditions are described elsewhere.

CROSS-REACTION

The 'cross-reactivity' of the antiserum with the various members of the tricyclic antidepressant group of drugs depends upon the label used and pH of the assay buffer as well as upon the particular antiserum bleed number.

Cross-reactivity studies carried out with antiserum bleed number 15/1/76 and using either ^3H -amitriptyline or ^3H -imipramine as labels are shown in Table 1. It can be seen that amitriptyline and nortriptyline have equal ability to displace ^3H -imipramine label from binding to the antiserum. This is fortunate, from a clinical point of view, since amitriptyline is partially *N*-demethylated within the body to produce nortriptyline which generally circulates at roughly equal concentrations to the parent drug. Since both of the compounds are biopharmacologically active the ability to measure 'total plasma tricyclics' on a single sample is advantageous. Comparisons of the results so obtained against those obtained by summing the peaks for amitriptyline and nortriptyline on GLC fractionation and quantitation are shown in Fig. 2.

It emerges from the cross-reactivity studies that alterations to the ring-structure by the insertion of an hydroxyl-group at position C_{10} virtually abolishes the ability of the compound to displace label from binding sites on the antiserum. This is

Table 1. Cross-reactivity (CR) of various tricyclic antidepressant drugs and their metabolites with an antinortriptyline antiserum.

Substance tested	Commercially available	³ H-amitriptyline label		³ H-imipramine label	
		ED ₅₀ ^a	% CR	ED ₅₀ ^a	% CR
Amitriptyline	+	0.4 ng	150	0.4 ng	100
Nortriptyline	+	0.6 ng	100	0.4 ng	100
Imipramine	+	1.0 ng	60	0.4 ng	100
Doxepin	+			0.3 ng	125
Protriptyline	+			0.4 ng	100
Desipramine	+	1.2 ng	50	0.7 ng	57
Desmethylnortriptyline	-	3.7 ng	16	3.1 ng	13
Amitriptyline N-oxide	-	31.0 ng	2	5.1 ng	7
10-Hydroxyamitriptyline	-	104.0 ng	<1	32.0 ng	1

^aAmount of substances needed to displace 50% of label.

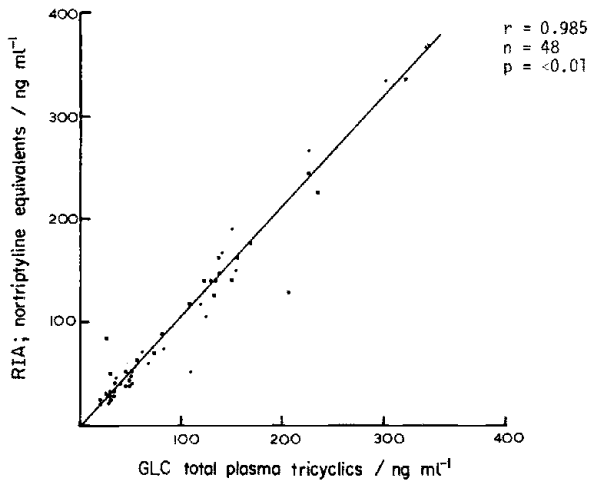


Fig. 2 Comparison of plasma tricyclic concentrations measured by GLC and RIA in patients treated with either nortriptyline or amitriptyline.

clinically relevant since ring-hydroxylation is important metabolically — the substances so produced being pharmacologically inactive.

DISCUSSION

The antidepressant pharmacological properties of the tricyclic group of drugs appears mainly to be determined by the presence of an unsubstituted tricyclic nucleus. Substitutions in the side chain seem to determine potency. Metabolic conversions occur

at both sites but those involving the ring structure are always associated with loss of pharmacological activity whereas those involving the side chain are more variable in their effects.

The ability of the antiserum raised in S259 to react specifically with diverse members of the tricyclic group enables assays to be set up for each one of them using the same ³H-imipramine label providing the appropriate compound (drug) is used to construct the calibration curve. Interference from other drugs not possessing the tricyclic nucleus does not occur.

Lack of parallelism between the different tricyclic drugs in the assay using ³H-imipramine precludes its use for measuring one member of the group in the presence of another without prior separation except in the case of amitriptyline and nortriptyline which cross-react identically. This is seldom a problem in practice, however, since it is unusual to use two tricyclic antidepressant drugs simultaneously in the same patient.

Increased, virtually absolute, specificity can be achieved, should it be required, using RIA to detect and quantitate and HPLC to separate the various cross-reacting substances along lines used by the authors to quantify cannabis products in blood and urine. Such a system retains the exquisite sensitivity and inherent group specificity of immunoassays as well as incorporating the high resolution separative capacity of HPLC. It suffers, however, from the disadvantage, for routine purposes, of cumbersomeness.

An immunoassay for desmethylinipramine was described by Spector *et al.* (1975) using an antiserum which showed similar cross-reactivity characteristics to antiserum S259 - namely, virtual extinction of binding by substitutions in the nuclear ring structure, and relatively little effect by major substitutions in the side chain. These findings are in accord with the dictum of Landsteiner (1945) that antibody specificity for haptens is directed mainly towards those antigenic determinants that are farthest removed from its link to the conjugating protein.

SUMMARY

A radioimmunoassay for tricyclic antidepressant drugs in plasma has been developed and evaluated. It employs an antiserum raised in sheep against a nortriptyline-BSA conjugate and uses ³H-imipramine as label. The assay uses unextracted plasma and is sensitive to 1 ng nortriptyline per ml plasma with a between-batch CV of less than 10%. The results obtained using the RIA correlate well with those obtained by GLC.

ACKNOWLEDGEMENTS

We should like to thank Dr R. Braithwaite for giving us plasma samples in which he had determined total tricyclic antidepressants by GLC.

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DISCUSSION

D. SAMPSON: Does the adjuvant used have an effect on the specificity of the antiserum produced? Do the separation methods affect the cross reactivity and does pH have any effect on cross reactivity?

V. MARKS: Firstly, the answer to the last question is that the pH at which the incubation is carried out does seem to have quite a marked effect upon the relative cross reactivities of the two tricyclics. We do not have any reason to believe that different adjuvants affect specificity of the antisera. Nonetheless some adjuvants, for example our own Marcol 52, seem to have certain advantages over others, such as Freund's, in so far as they reduce ulceration without diminishing immunological response. Finally we are quite convinced on the basis of our own experimental work that different separation methods do have profound effects upon both the specificity and sensitivity of immunoassay methods.

P. STANLEY: Do you know of any evidence which suggests that amitriptyline and nortriptyline are equipotent pharmacologically?

V. MARKS: I am not aware of any convincing evidence that amitriptyline and nortriptyline have different biological potencies but opinions differ.