Biochemical Pharmacology, Vol. 26, pp. 353–354. Pergamon Press, 1977. Printed in Great Britain.

A method for the determination of platelet and plasma monoamine oxidase in whole blood

(*Received* 9 *March* 1976; *accepted* 16 *July* 1976)

Clinical improvement of patients receiving phenelzine (Phe) was shown to correlate positively with the degrees of monoamine oxidase (monoamine: O_2 oxidoreductase (deaminating), EC 1.4.3.4.) (MAO) inhibition [1, 2] and, in particular, platelet MAO [3]. Dunleavy *et al.* [4] claimed that only when REM sleep is abolished can phenelzine effect improvement. The dose required to achieve this state is 90 mg/day, twice that usually administered to patients. Data from animal studies [5] showed that small doses of phenelzine have a cumulative effect on MAO, which is never as great as that observed after a single high dose of the drug.

It is possible that some patients receiving Phe, or some other MAOI, never attain the level of MAO inhibition necessary for effective therapy. For this reason, it may be important to assay enzyme activity at frequent intervals at the beginning of therapy in order to adjust the dosage to attain a high level of exnzyme inhibition and thus shorten the time course of treatment.

Robinson *et al.* [6] detailed the characteristics of the two MAOs in blood and described methods for assaying the activity in platelet and plasma prepared from fresh blood.

We present evidence to suggest that platelet and plasma MAO are represented respectively by the activity in the pellet and supernatant fractions of lysed whole blood prepared by high speed centrifugation.

MATERIALS AND METHODS

Radioactively [^{14}C]-labelled benzylamine was obtained from Radiochemical Centre, Amersham. Pargyline and clorgyline were gifts from Abbott Laboratories and May and Baker respectively.

Preparation of platelets and plasma. Blood (20 ml) was withdrawn from the antecubital vein, added to 1% EDTA in physiological saline (2 ml) in a siliconised tube and centrifuged at 500 *g* for 20 min. The platelet-rich plasma was aspirated and centrifuged at 2000 *g* for 10 min. The platelets formed a firm pellet and the plasma could be decanted without contamination. The platelets were resuspended in a volume of 0.2 M phosphate buffer, pH 7.2, equivalent to the original volume of platelet-rich plasma. These preparations were kept at −20° until required.

Preparation of pellet and supernatant fractions Fresh blood (20 ml) was added to 1% EDTA in physiological saline (2 ml) and lysed by freezing and thawing. The lysate was centrifuged at 17,000 *g* for 30 min at 0°, the supernatant decanted and the pellet resuspended in a volume of 0.2 M phosphate buffer, pH 7.2 equivalent to the original volume of whole blood. These preparations were kept at −20° until required.

Assay of monoamine oxidase activity The method used was that described by Robinson *et al.* [6] for plasma. In order that comparisons could be made we adopted the same technique for platelets. At the end of the incubation period of 1 hr at 37°, cold 60% PCA (0.15 ml) was added, and the radioactively-labelled product of MAO activity was extracted into toluene (3 ml) by shaking, followed by centrifugation. Samples, together with duplicate blanks in which PCA had been added at the beginning of the incubation, were assayed in duplicate. Two aliquots of the organic upper layer were removed, added to 10 ml of toluene-based phosphor containing PPO, dimethyl POPOP and Triton-X100 [7] and counted in a Packard Tricarb scintillation counter.

RESULTS AND DISCUSSION

The values for control levels of MAO activity in whole blood, platelet, plasma, pellet and supernatant, calculated in terms of the volume of whole blood from which these fractions derive, are indicated in Table 1.

Addition of MAO activity in pellet to that in supernatant gives a value which accounts completely for the whole blood activity.

K_m values for benzylamine oxidation by pellet (1.8×10^{-4} M) and platelet (1.5×10^{-4} M) are similar, but different from the values obtained from plasma (3×10^{-4} M) and supernatant (3.4×10^{-4} M). These K_m values for platelet and plasma enzymes agree with the values detailed by Robinson *et al.* [6] and confirm the existence of two MAOs in blood. That the K_m values for platelet and pellet are similar as are those from plasma and supernatant, suggest that the techniques of high speed centrifugation has also separated the two enzymes. This is further supported by the inhibitor studies.

The oxidation of benzylamine by the enzyme contained in platelet is totally inhibited by pargyline (10^{-4} M) a known inhibitor of MAO type A. Clorgyline inhibits both the A and B forms of MAO but different levels of sensitivity. The ID_{50} of clorgyline for benzylamine oxidation by MAO type A (Murphy and Donnelly, 1974) is similar to that found for the inhibition of platelet enzyme (8.0×10^{-6} M) in the present study. Semicarbazide, on the other hand, has no effect on the platelet enzyme.

Similar data were obtained for the oxidation of benzylamine by the enzyme contained in the resuspended pellet suggesting that the platelet and pellet enzymes are similar, probably both type A and not type B.

Table 1. MAO Activities in whole blood fractions

Sample No.	Total control whole blood	Pellet	Supernatant	Total control pellet and supernatant	Platelet	Plasma	Total control platelet and plasma
1	13.14	11.18 (70)	4.84 (30)	16.02	6.6 (71)	2.65 (29)	9.25
2	16.71	13.24 (70)	5.58 (30)	18.82	8.3 (71)	3.4 (29)	11.7

Benzylamine oxidised, nmoles/hr/ml whole blood from which fraction is derived. The figures in parentheses are the activity expressed as a percentage of the corresponding total control.

Plasma or supernatant MAO activity is inhibited by semicarbazide and is not affected by either pargyline or clorgyline. These results indicate that the enzymes contained in plasma and supernatant are similar to one another, different from the enzymes contained in platelet and resuspended pellet, and confirm that plasma MAO is neither type A nor B (Murphy and Donnelly, 1974).

Although Robinson *et al.* [6] recommend that the platelet enzyme be monitored during MAOI therapy, they compared urinary metabolites with both blood MAO activities during administration of MAOIs to normals only and not to patients suffering from psychiatric disorders.

Youdim *et al.* [11] suggested that because isoenzymes have different inhibitor kinetics, any one inhibitor may have a particular mechanism of action dependent on the isoenzyme it inhibits most strongly. Controlling both platelet and plasma MAO would, therefore, appear to be a necessary requisite when investigating the biochemical correlates of clinical improvement induced by MAOIs.

The comparisons made here suggest that particle bound and soluble enzyme activities may be equated with platelet and plasma activities respectively. In high speed centrifugation, we have a technique which, in practice, provides a clinically more convenient method for the assay of both blood monoamine oxidases.

Acknowledgement—We wish to thank Mrs. M. Armstrong for excellent technical assistance.

Department of Psychological Medicine,
University of Newcastle upon Tyne,
Newcastle upon Tyne NE2 4LP,
England

ELIZABETH F. MARSHALL
IAIN C. CAMPBELL

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