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THE EFFECT OF THREE MONOAMINE OXIDASE INHIBITORS ON HUMAN PLASMA MONOAMINE OXIDASE ACTIVITY*

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Abstract—The effect of a single oral dose of three monoamine oxidase inhibitors, Marplan [1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine], Parnate sulfate (cis-trans-phenylcyclopropylamine sulfate) and Nardil (β-phenylethylhydrazine) on human plasma monoamine oxidase was determined by the radioassay of Otsuka and Kobayashi, *Biochem. Pharmac.* **13**, 995 (1964). Parnate was the only drug that effected definite inhibition of the plasma enzyme. It was suggested that plasma monoamine oxidase titers can be used to monitor *in vivo* the effectiveness of monoamine oxidase inhibitor drugs.

THE association of monoamine oxidase (MAO) with depression has been claimed as a result of numerous studies of monoamine oxidase inhibitors.[1] This subject has been well reviewed by Biel *et al.*[2] and Zirkle and Kaiser.[3] However, there is some disagreement about the mechanism and nature of this association.[4] The beneficial clinical effects of monoamine oxidase inhibitor drugs appear to be well enough established to stimulate continued interest in this class of drugs. Unfortunately, their clinical use has been complicated by reported incidences of untoward responses.[5, 6]

The observation of MAO activity in human plasma[7] suggested the possibility of using plasma in evaluating the effectiveness of a MAO inhibitor drug *in vivo*. Plasma titers of MAO might serve to monitor the drug's effectiveness in the peripheral circulation. This paper presents the results of the first human experiments designed to test this thesis with three MAO inhibitor drugs: Parnate sulfate, Nardil, and Marplan.

METHODS AND MATERIALS

Radioactive material

^{14}C-Tyramine, 5·78 mc/mmole, was purchased from New England Nuclear Corp. This was diluted with nonisotopic tyramine to give a solution with an activity of 100,000 dis/min per 5 μg tyramine (free base) per 0·1 ml solution.

Monoamine oxidase inhibitor drugs

All drugs used were given orally in a single dose. Marplan (Roche), 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine, 30 mg; Parnate sulfate (Smith Kline & French), cis-trans-phenylcyclopropylamine sulfate, 20 mg; and Nardil (Warner-Chilcott), β-phenylethylhydrazine, 30 mg.

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Sample collection and assay

Ten ml blood was withdrawn from each subject by an oxalated Vacutainer (Becton, Dickinson & Co.). The blood was centrifuged and the plasma removed. Two ml plasma was then analyzed for MAO activity as previously described.[7] Each assay tube contained 2 ml oxalated plasma, 5 μg ^{14}C-tyramine, and a final concentration of 10^{-5} M aminoguanidine and 10^{-3} M EDTA (disodium salt) in a final volume of 2·3 ml. Incubation time was 2 hr in air at 37°. Anisole-PPO was used to extract the radioactive end product.

All radioassays were done in a Nuclear-Chicago model 725, or a Packard model 314-EX2, liquid scintillation counter. The counting efficiency was approximately 50%. Under these conditions, 10,000 counts/min was equivalent to 1 μg tyramine metabolized by plasma.

EXPERIMENTAL

An estimate of normal male plasma monoamine oxidase titer was made by averaging values of twenty subjects. It was found to be 1502 counts/min with a standard deviation of 1672 counts/min. Nineteen values were between 272 and 2714 counts/min. One value was 7808 counts/min. If the 7808 value was eliminated, the recomputed average was 1170 counts/min with a standard deviation of 807 counts/min.

TABLE 1. DAILY PLASMA MAO TITERS OF FIVE NORMAL MALE SUBJECTS ASSAYED FOR THREE CONSECUTIVE WEEKS

Day	Subject A	Subject B	Subject C	Subject D	Subject E
1	1532	4644*	1372	1756	1920
2	813	408	1282	1664	3403
3	402	483	615	1170	1109
4	734	612	695	1118	2040
5	*904*	*552*	*669*	*940*	*1832*
8	1272	916	831	2475	1111
9	1600	279	571	984	1562
10	1594	1009	706	1366	2534
11	796	602	725	1691	854
12	*1209*	*911*	*4138**	*805*	*659*
15	869	1098	772	1724	893
16	667	606	300	449	799
17	773	289	554	1203	1447
18	410	438	445	1060	558
Avg. MAO	970	918	977	1315	1480
S.D.	±406	±1102	±955	±509	±806
*Adjusted avg.		631	734		
S.D.		±270	±299		

Results in counts/min/2 ml plasma.

* Omitted and average and standard deviation recomputed.

In order to ascertain the daily variation of plasma MAO titers, five normal male subjects were followed once daily for 3 consecutive weeks. The data are presented in Table 1. Subjects B and C had one relatively high value during the test period (i.e. in excess of three times the standard deviation). If these values were eliminated, the standard deviations of the recomputed means were 43 and 41 per cent, respectively. These standard deviations were then comparable to the other three values which were 42, 39 and 55 per cent, respectively, for Subjects A, D and E.

Three normal male volunteers were given a single oral dose of 20 mg Parnate sulfate, a nonhydrazine MAO inhibitor. Blood samples were taken at 0, 15 min, 30 min, 1 hr, 1·5 hr, 2 hr, 3 hr, 4 hr, 7 hr, 24 hr, and again at 5 days. The results are shown in graph A of Fig. 1. It can be seen that all subjects showed complete inhibition of MAO activity within 3 hr after drug treatment and were still inhibited after 24 hr. All subjects showed an elevated plasma MAO titer after 5 days.

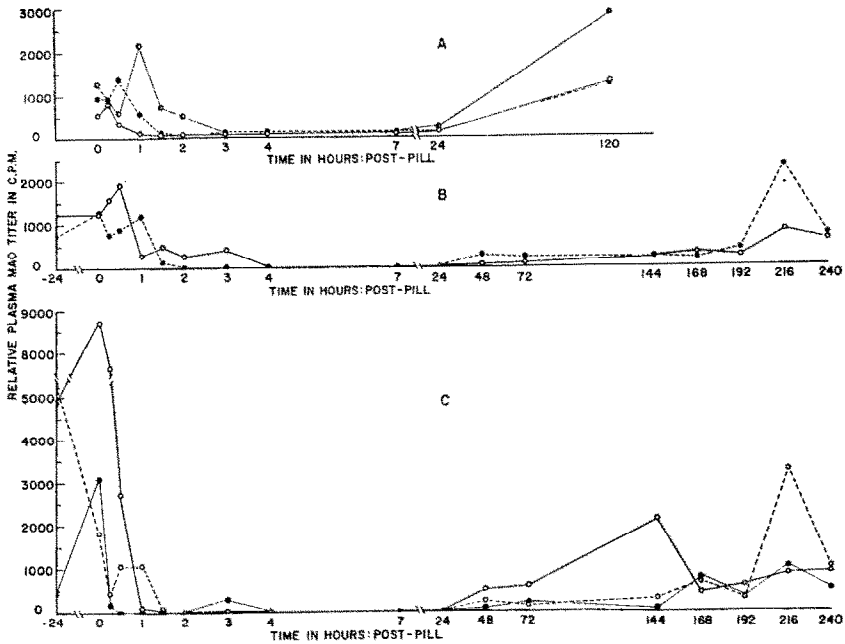

FIG. 1. Pattern of relative plasma MAO titers of 8 normal male subjects after a single 20-mg oral dose of Parnate sulfate. Each point represents the average value of duplicate determinations of a single plasma sample taken at the indicated time.

A second run with Parnate sulfate was made almost 1 yr later on five normal male subjects. This time a plasma sample was taken 24 hr before the experiment and also at more frequent 24-hr intervals after drug treatment. The data are presented in graphs B and C of Fig. 1. It can be seen that four out of five subjects showed complete inhibition of MAO within 2 hr after taking the drug. The actual plasma MAO titers were less than 50 counts/min/2 ml plasma. Again four out of five subjects appeared to have depressed plasma MAO levels 7 days after a single dose of Parnate sulfate. In graph C, volatility of the plasma MAO index is illustrated by the large change in titer observed between minus 24 hr and zero time. In spite of these large variations, this drug effectively inhibited plasma MAO in all individuals tested for at least 72 hr. The acute rise in enzyme activity observed on day 9 (216 hr) after drug treatment is reminiscent of the "rebound phenomenon" generally associated with physiological response to inhibitors.

Parnate sulfate was now given to three acute male schizophrenic subjects. The results are presented in Fig. 2. One subject appeared to resist the effect of the drug, as indicated by a rise in MAO titer at 7 hr after treatment. However, after 24 hr, this subject's

plasma titer was almost zero. It should be noted that a single dose of Parnate sulfate was effective for 72 hr.

Nardil, a hydrazine-type MAO inhibitor, was given to four normal male subjects in a single oral dose of 30 mg, and their plasma MAO titers were followed as before.

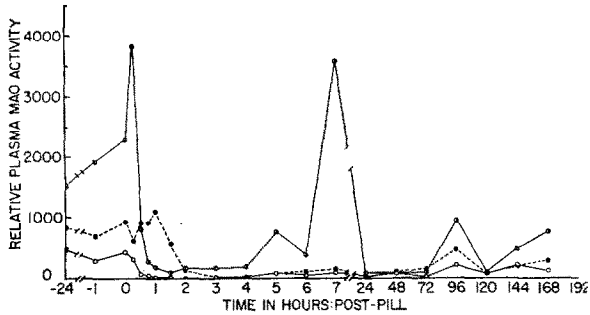

FIG. 2. Pattern of relative MAO titers of 3 schizophrenic male subjects after a single 20-mg oral dose of Parnate sulfate. Each point represents the average value of duplicate determinations of a single plasma sample taken at the indicated time.

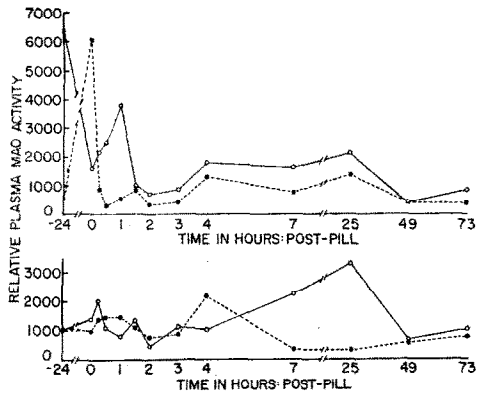

FIG. 3. Pattern of relative plasma MAO titers of 4 normal male subjects after a single 30-mg oral dose of Nardil. Each point represents the average of duplicate determinations of a single plasma sample taken at the indicated time.

The data are presented in Fig. 3. It was difficult to judge the effectiveness of the drug from these data. In the top graph, the two subjects showed a huge change in plasma MAO titer between minus 24 hr and zero time. Twenty-five hr after drug treatment, both subjects had relatively normal plasma levels of the enzyme. This was different from the Parnate sulfate experiment in which the enzyme level was almost zero at 24 hr after drug treatment. In the lower graph of Fig. 3, one subject had an elevated MAO titer after 25 hr; the other may have had a slightly lower than zero-time titer of MAO. No conclusion could be made from these data regarding Nardil's ability to inhibit plasma monoamine oxidase *in vivo*.

In Fig. 4, the data gathered on three acute schizophrenic male subjects given a single 30-mg oral dose of Nardil are presented. It can be seen that the response of these three subjects to Nardil was different from that observed in normals. Twenty-four hr after the administration of the drug, the plasma levels of MAO were elevated in all cases. Although large changes in plasma MAO levels were seen in untreated normal subjects, it must be recalled that these subjects received a single dose of Nardil, a MAO inhibitor. From these data on the three schizophrenic subjects, it was concluded that Nardil, in a single 30-mg oral dose, was not an effective plasma monoamine oxidase inhibitor.

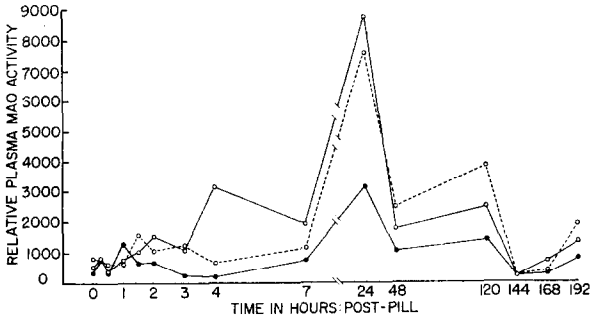

FIG. 4. Pattern of relative plasma MAO titer of 3 schizophrenic male subjects after a single 30-mg oral dose of Nardil. Each point represents the average value of duplicate determinations of a single plasma sample taken at the indicated time.

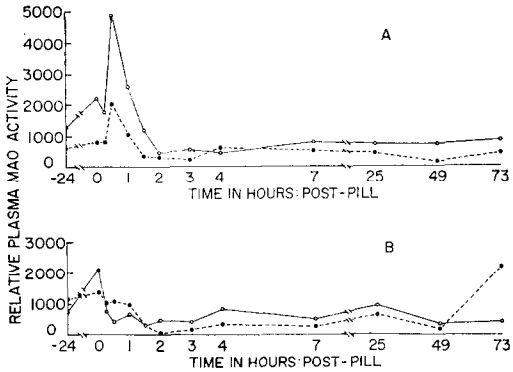

FIG. 5. Pattern of relative plasma MAO titer of 4 normal male subjects after a single 30-mg oral dose of Marplan. Each point represents the average of duplicate determinations of a single plasma sample taken at the indicated time.

In Fig. 5, the data obtained from four normal male subjects given a single oral dose of 30 mg Marplan are presented. No definitive conclusion was drawn from these data concerning the drug activity *in vivo* against monoamine oxidase. At 25 hr, all subjects had plasma titers ranging from 500 to 1000 counts/min/2 ml plasma.

Marplan was not tested on schizophrenic male subjects. However, the plasma titers of monoamine oxidase of six males and thirteen female patients at the Worcester State Hospital on Marplan therapy in excess of 30 days were determined. The data are

presented in Table 2. As a point of reference, twenty-two female patients at the hospital on non-MAO inhibitor drugs (Stelazine, Trilafon, or Artane) were also examined. Their average plasma monoamine oxidase titer was 1511 counts/min with a standard deviation of 755 counts/min. The values ranged from 538 to 3117 counts/min. Thus, these women on non-MAO inhibitor drugs had enzyme levels comparable to those of

TABLE 2. PLASMA MAO TITERS OF NINETEEN SCHIZOPHRENIC SUBJECTS ON MARPLAN THERAPY IN EXCESS OF 1 MONTH

Males		Females			
No.	MAO Titer	No.	MAO Titer	No.	MAO Titer
1	102	1	59	8	459
2	371	2	83	9	690
3	540	3	94	10	968
4	952	4	100	11	1383
5	1465	5	124	12	1867
6	1760	6	225	13	3258
		7	370		
Avg. = 865 ± 648		Avg. = 745 ± 942			

Results in counts/min/2 ml plasma.

normal males. The data in Table 2 show that at least four of the thirteen women and three of the six male patients on Marplan therapy had normal levels of plasma MAO. One must conclude from these data that Marplan has either become ineffective as an enzyme inhibitor in some subjects or that it may have been ineffective from the beginning.

TABLE 3. INHIBITION *in vitro* OF HUMAN PLASMA MAO BY MARPLAN, NARDIL, AND PARNATE*

Compound	Concentration of inhibitor				
	2×10^{-5}M	2×10^{-6}M	2×10^{-7}M	2×10^{-8}M	2×10^{-9}M
Control	0	0	0	0	0
Marplan	84	51	26		
Nardil		81	58	45	
Parnate			100	59	36

* Marplan and Nardil values are averages of three experiments. Parnate values are averages of two experiments. All values are expressed as per cent inhibition compared to control values. Conditions as described under Methods.

The relative effectiveness of Marplan, Nardil, and Parnate sulfate as MAO inhibitors *in vitro* was measured in pooled human plasma. Three different samples of pooled plasmas were used. It was found that Parnate sulfate was the most effective drug of the three tested. For approximately 50% inhibition, the concentration of Parnate required was approximately 10^{-2} M less than Marplan and about 10^{-1} M less than Nardil (Table 3).

DISCUSSION

The classification of plasma monoamine oxidase is difficult because there is no suitable convention to follow in this field.[8, 9] Plasma amine oxidase activity isolated from bovine plasma by Tabor *et al.*[10] and by Yamada and Yasunobu[11] have little to no activity against the usual biogenic monoamines and are inhibited by carbonyl reagents. Tabor *et al.* have classified their preparation as spermine oxidase; Yamada and Yasunobu call their preparation monoamine oxidase. However, the preparation of Yamada and Yasunobu has been classified as spermine oxidase by Blaschko.[8] Recently, McEwen[9] has purified human plasma MAO approximately 5000-fold. Benzylamine was routinely used as the substrate. The purified enzyme was found to oxidize the amines tyramine, dopamine, 3-methoxydopamine, tryptamine, kynuramine, ethanolamine, and β-mercaptoethylamine, but not as actively as benzylamine. McEwen reported that only the catecholamine derivatives that lacked a β-hydroxyl group were deaminated. The purified human monoamine oxidase was similar to bovine spermine oxidase in that both enzymes were completely inhibited by semicarbazide and strongly inhibited by isoniazid and iproniazid. The plasma monoamine oxidase assay described in this paper is routinely carried out with tyramine as substrate in the presence of sufficient aminoguanidine, a carbonyl reagent, to inhibit completely any diamine oxidase activity.[7] During the development of this assay,[7] it was found that less than 26 per cent inhibition of human plasma MAO was observed in the presence of 10^{-4} M semicarbazide.

In view of these facts, it seemed unlikely that McEwen's purified human plasma monoamine oxidase, which is very sensitive to semicarbazide, was the same as that measured by our assay. In terms of the relation of the plasma enzyme measured by us to tissue monoamine oxidase activity, the following comments can be made. Tissue monoamine oxidase[8] is considered to be a particulate enzyme, insensitive to carbonyl reagents and sensitive to hydrazine derivatives. Tyramine is a model substrate for tissue monoamine oxidase. Plasma monoamine oxidase, as measured in this paper, is assayed with tyramine as substrate, and the enzyme activity has been found to be insensitive to carbonyl reagents and to show a sensitivity to hydrazine derivatives similar to that of tissue enzymes. Therefore, it is our contention that the plasma MAO assay carried out in the presence of aminoguanidine, with tyramine as substrate, is measuring monoamine oxidase activity which is similar to that found in tissues.

The data presented show that there is a wide distribution of normal values for plasma MAO, and that these values are highly variable from day to day. In spite of these variations, a single dose of Parnate sulfate caused a definite inhibition of the plasma enzyme. Similar data obtained on Nardil and Marplan, however, were inconclusive. The observations *in vitro* (Table 3) suggest that the lack of a definitive response to a single oral dose of either Marplan or Nardil, *in vivo*, might be attributable to insufficient dosage. The concentration of Parnate sulfate required to inhibit plasma monoamine oxidase approximately 50 per cent *in vitro* was about 10^{-2}M less than Marplan and about 10^{-1} M less than Nardil.

The data presented on Parnate sulfate, Figs. 1 and 2, show that a single oral dose of 20 mg inhibited plasma MAO within 2 hr and that the inhibition persisted for at least 3 days. These observations suggest that the standard dose schedule currently recommended for Parnate sulfate, which is 20 mg daily for 2–3 weeks, may be excessive if the objective of the drug therapy is monoamine oxidase inhibition.

The therapeutic effects of MAO inhibitor drugs are attributed to their action in the brain.[1] Since a relationship between brain and plasma MAO has not been established, the value of plasma MAO determinations has been rationalized as follows. First, it was noted that all three drugs used in these studies have been shown by others to inhibit, *in vivo*, the brain, liver, and intestinal monoamine oxidases of animals.[1-3] These drugs are effective inhibitors of MAO obtained from any tissue if used in sufficient concentration. The effectiveness of Marplan, Nardil, and Parnate in inhibiting human plasma MAO *in vitro* has been demonstrated in Table 3. Second, it was assumed that the drug can be transported to the brain only via the circulation. Therefore, it would appear reasonable that the inhibition of the blood enzyme would precede any inhibition of the brain enzyme. If plasma MAO inhibition is observed after drug treatment, it can be surmised that the drug is available for action in the brain via the blood stream. Whether or not the drug penetrates the blood–brain barrier in humans cannot be ascertained by any method at this time. On the other hand, if no inhibition of plasma enzyme is observed, any action in the brain by the drug treatment would seem unlikely.

In conclusion, it is hoped that the plasma monoamine oxidase assay may prove to be another useful method that can measure, *in vivo*, the effect of monoamine oxidase inhibitor drugs.

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