

Niacin depletion in Parkinsonian patients treated with L-dopa, benserazide and carbidopa

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Summary

1. Benserazide and carbidopa, decarboxylase inhibitors used in the treatment of Parkinson's disease, have been shown to inhibit the enzyme kynurenine hydrolase in rat and mouse liver. This results in reduced synthesis of nicotinamide coenzymes from tryptophan, and hence an increased reliance on dietary niacin.

2. Pellagra might be expected as a result of this inhibition of endogenous synthesis of nicotinamide nucleotides, but has not been reported in patients treated with either drug.

3. The urinary excretion of *N*¹-methyl-nicotinamide, a product of nicotinamide nucleotide metabolism, is considerably reduced in patients treated with dopa alone or in combination with an inhibitor of peripheral dopa decarboxylase, to as low as 40% of the control value. This means that many of these patients could be classified as 'at risk' of niacin deficiency, even if not frankly deficient.

4. Patients treated with dopa plus a decarboxylase inhibitor, but not those treated with dopa alone, also show a reduced excretion of xanthurenic acid, and an increased excretion of kynurenine, as would be expected after inhibition of the kynurenine pathway, and possibly indicative of marginal vitamin B₆ deficiency.

Key words: bromocriptine, dihydroxyphenyl- α -hydrazino- α -methylpropionic acid, L-dopa,

kynurenic acid, kynureninase, kynurenine, *N*¹-methylnicotinamide, nicotinamide deamidase, Parkinson's disease, pellagra, seryl trihydroxybenzylhydrazine, xanthurenic acid.

Abbreviation: NAD, NADP, nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate respectively.

Introduction

The dopa decarboxylase inhibitors benserazide [Ro4-4602; *N*-(seryl)-*N'*-(2,3,4-trihydroxybenzyl)hydrazine] and carbidopa [MK 486; β -(3,4-dihydroxyphenyl)- α -hydrazino- α -methylpropionic acid] are potent inhibitors of the liver enzyme kynurenine hydrolase (L-kynurenine hydrolase, EC 3.7.1.3) (Bender, Smith & Humm, 1977; Bender & Smith, 1978). Kynurenine hydrolase catalyses the hydrolysis of hydroxykynurenine to hydroxyanthranilic acid in the oxidative pathway of tryptophan metabolism (see Fig. 1). Quantitatively minor products of this pathway are the coenzymes NAD and NADP. Significant inhibition of kynurenine hydrolase would therefore be expected to lead to a reduced ability to synthesize NAD from tryptophan, and hence to an increased dependence on dietary sources of the vitamin niacin, and possibly to development of pellagra. Clinical pellagra has not been reported as a side effect of therapy with benserazide or carbidopa; in the present study serum and urine samples from patients treated with these drugs have been investigated in an attempt to detect sub-clinical niacin depletion.

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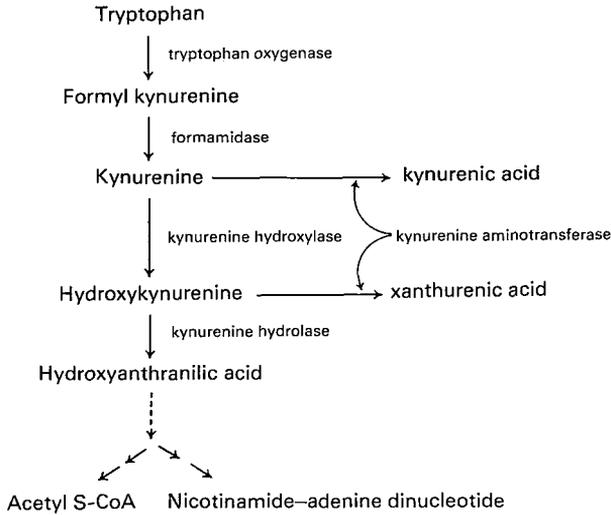


FIG. 1. The oxidative pathway of tryptophan metabolism.

Patients and methods

Patients with Parkinson's disease attending The Middlesex Hospital and University College Hospital were asked to provide a random sample of urine and a sample (10 ml) of venous blood. The blood was allowed to clot and serum was separated and frozen. Urine and serum samples were stored at -20°C until required for analysis; no preservative was added to the urine samples.

Depending on treatment, the patients were divided into three groups, as shown in Table 1. Seven were receiving the dopaminergic agonist bromocriptine, ten relatively high doses of L-dopa and 32 were receiving lower doses of dopa together with one of the inhibitors of peripheral dopa decarboxylase (aromatic L-amino acid decarboxylase, EC 4.1.1.28), benserazide or carbidopa. Of these patients, 26 were receiving Sinemet (carbidopa + dopa, Merck, Sharp and Dohme) and the remaining six were receiving Madopar (benserazide + dopa, Roche). As far as is known, none of the patients was receiving any other medication, and none was known to be taking any vitamin preparation. Two groups of subjects were used as control: 13 male medical students and members of research staff and 19 perimenopausal women attending the menopause clinic at The Hospital for Women, Soho (London W.1). None of the control subjects was known to have any neurological disease and none was receiving any drugs that might be expected to affect tryptophan and niacin metabolism. Patients who complained of depres-

sion were excluded from the control group since they might be expected to have abnormal tryptophan metabolism (Coppen, Eccleston & Peet, 1973).

Serum total tryptophan concentration was determined by a modification of the norharman fluorescence method of Denckla & Dewey (1967), with perchloric acid rather than trichloroacetic acid, and the extent of tryptophan binding to serum albumin was determined by the small-scale equilibrium dialysis method of Bender, Boulton & Coulson (1975).

N^1 -Methylnicotinamide was determined in urine by a small-scale modification of the alkali-ketone fluorescence method of Carpenter & Kodicek (1950). Xanthurenic acid and kynurenic acid and kynurenine were determined in urine after ion-exchange column chromatography on small columns of Dowex 50W (H^+) resin, by a modification of the method of Satoh & Price (1958) as described previously (Bender *et al.*, 1977). Xanthurenic acid and kynurenic acid were determined fluorimetrically (Satoh & Price, 1958), and kynurenine colorimetrically after diazotization and coupling to naphthyl ethylenediamine, modified from the method of Joseph & Risby (1975). Because the urine samples were collected randomly, it was not possible to express concentrations per total urine volume, and therefore all data have been expressed per mol of creatinine, determined by the alkali-picrate method (Oser, 1965).

The activity of nicotinamide deamidase (nicotin-

amide amidohydrolase, EC 3.5.1.19) was determined by a modification of the method of Gadd & Johnson (1974). Commercially available spray-dried cells of *Micrococcus lysodeikticus* were used as the source of the enzyme, without any attempt at purification. The cells were suspended in potassium chloride solution (0.15 mol/l) to give a concentration of 80 µg/ml; the incubation mixture contained 100 µl of this bacterial suspension (8 µg of cells), 100 µl of sodium phosphate buffer (0.11 mol/l), pH 8.2, and 100 µl of [*carbonyl*-¹⁴C]nicotinamide solution (19 µmol/l). Inhibitors were added to the buffer, and pre-incubated with the enzyme preparation for 10 min at 30°C before the reaction was initiated by addition of the substrate. After a further 10 min incubation, the reaction was stopped by the addition of 100 µl of hydrochloric acid (5 mol/l) containing approximately 1 mg/ml each of non-radioactive nicotinamide and nicotinic acid. After centrifugation to remove precipitated protein, 25 µl aliquots of the supernatant were applied to the origins of paper chromatograms, which were developed in acetone/propan-2-ol/water/aq.-ammonia solution (50:40:7:3, by vol.). The regions corresponding to substrate and product were located under ultraviolet light, cut out and radioactivity was measured by liquid-scintillation spectrometry as described previously (Bender & Coulson, 1972).

Comparisons were made by unpaired *t*-test.

Results

The two groups of control subjects, healthy young men and perimenopausal women, showed no significant differences in any of the variables measured, and results from these two groups have therefore been combined to give a single 'control' group (Table 1). There was no difference between the two groups in any of the biochemical variables and there was no evidence of any age-related change in the variables within each group. Similarly, there were no significant differences between the small group of patients receiving benserazide + dopa and the larger group receiving carbidopa + dopa. Results for these two groups of patients have therefore been combined in Table 1.

There were no significant differences in serum total tryptophan between any of the three groups of patients and the control subjects (Table 1). Serum diffusible tryptophan values (as an index of albumin binding of tryptophan) were widely scattered, with no differences between the groups of subjects, but with a very large within-group standard deviation. This is presumably because the patients attended the clinics at different times of the day; some were fasting and some recently fed, so that serum non-esterified fatty acid concentrations would therefore vary widely between patients, and this would have a considerable effect on tryptophan binding to albumin (Curzon, Friedel & Knott, 1973).

TABLE 1. Details of subject groups involved in the study and serum tryptophan and urine tryptophan metabolite concentrations

Figures show either median value with range in parentheses or mean \pm SD. Madopar: benserazide + L-dopa (Roche); Sinemet: carbidopa + L-dopa (Merck, Sharp and Dohme). *P* values (by *t*-test): * significantly different from control ($P < 0.001$); ** marginally significantly different from control ($0.02 > P > 0.01$).

	Control (<i>n</i> = 32)	Bromocriptine (<i>n</i> = 7)	L-Dopa (<i>n</i> = 10)	Madopar or Sinemet (<i>n</i> = 32)
Age (years)	13 males 32(18-55) 19 females 45(35-58)	58(40-67)	60(57-71)	63(55-70)
Duration of illness (years)	—	3(2-5)	12(8-14)	5(4-16)
Duration of present therapy (years)	—	3(1-2)	7(5-9)	5(2-8)
Dose (mg/day)	—	60(20-100)	4000 (2250-5000)	500(200-1000)
Serum tryptophan (µmol/l)	57.8 \pm 7.9	59.9 \pm 12.7	52.8 \pm 19.5	56.9 \pm 15.9
Urine concentrations (mmol/mol of creatinine):				
N ¹ -methylnicotinamide	2.1 \pm 0.8	2.4 \pm 0.5	0.8 \pm 0.5*	0.8 \pm 0.4*
Xanthurenic acid	11.3 \pm 6.1	12.9 \pm 6.2	11.2 \pm 5.4	4.8 \pm 4.5*
Kynurenic acid	6.5 \pm 3.8	1.9 \pm 1.2**	3.9 \pm 2.1	4.1 \pm 3.2
Kynurenine	0.6 \pm 0.3	1.9 \pm 0.9**	0.6 \pm 0.7	1.4 \pm 1.3*

Urine N^1 -methylnicotinamide concentration was significantly lower ($P < 0.001$) in patients treated with dopa or with dopa + decarboxylase inhibitor than in either the control subjects or patients treated with bromocriptine. Urine xanthurenic acid was significantly lower ($P < 0.001$) in the decarboxylase inhibitor-treated patients than in any of the other groups; kynurenic acid was unaffected except in the small group of bromocriptine-treated patients, in whom it was somewhat lower than in the control subjects, although not significantly different from the concentration found in other groups of patients. Urine kynurenine concentration was significantly elevated in the bromocriptine ($0.02 > P > 0.01$) and decarboxylase-inhibitor ($P < 0.001$) treated patients compared with the control subjects or those receiving dopa alone.

Discussion

The reduced xanthurenic acid and kynurenine excretion in the benserazide and carbidopa-treated patients may be explained by the inhibition of kynurenine hydrolase together with inhibition of kynurenine aminotransferase (L-kynurenine-2-oxoglutarate aminotransferase (cyclizing); EC 2.6.1.7); such inhibition has been observed in liver from benserazide-treated mice. Increased kynurenine excretion would be expected if its onward metabolism were inhibited, and in mice it has been shown that benserazide administration leads to an increase in the concentration of kynurenine in the liver (Bender *et al.*, 1977). The changes in kynurenic acid and kynurenine excretion in the bromocriptine-treated patients cannot be explained.

Kynurenine hydrolase is a pyridoxal phosphate (vitamin B₆)-dependent enzyme, and it is well established that dopa will form a biologically inactive adduct with pyridoxal phosphate (Fellman & Roth, 1971). Acute administration of dopa to experimental animals at doses similar to those used in the treatment of Parkinson's disease has been shown to lead to a significant depletion of tissue pyridoxal phosphate (Kurtz & Kanfer, 1971), although Yahr & Duvoisin (1972) have reported no difference in plasma pyridoxal phosphate concentration between dopa-treated and control patients. The reduced N^1 -methylnicotinamide excretion that we found in dopa-treated patients might reflect a moderate degree of vitamin B₆ depletion. If this is so, it is surprising that xanthurenic acid and kynurenic acid and

kynurenine excretion are unchanged in patients receiving dopa alone, especially since Cozzolino, Campriani & Campanelli (1975) reported that dopa administration to Parkinsonian patients led to an increase in kynurenine excretion (and in the excretion of anthranilic acid and hydroxykynurenine) with a decrease in the excretion of hydroxyanthranilic acid, indicative of inhibition of kynurenine hydrolase. They did not measure xanthurenic acid and kynurenic acid, or nicotinamide metabolites.

The most striking finding in our study is the very low excretion of N^1 -methylnicotinamide in patients treated with dopa, either alone or in combination with a decarboxylase inhibitor (about 40% of that in control subjects). By the standards used in field studies to determine niacin nutritional status, seven of the patients treated with dopa + inhibitor and five of those treated with dopa alone would be classified as niacin deficient, excreting less than 0.5 mmol of N^1 -methylnicotinamide/mol of creatinine. The other five dopa-treated patients, and five of the remaining dopa + inhibitor-treated patients, excreted more than 1 mmol of N^1 methylnicotinamide/mol of creatinine, which is within the normal range. This leaves 20 out of the 32 patients treated with dopa + an inhibitor of peripheral dopa decarboxylase in a group that would be classified as 'borderline deficient' or 'at risk' of niacin deficiency (Gontzea, Rujinski & Sutzesco, 1976). It is possible that a poor diet may be a factor in the low excretion of N^1 -methylnicotinamide in these patients; however, in those patients treated with bromocriptine, N^1 -methylnicotinamide excretion was normal. There was no significant correlation between the excretion of N^1 -methylnicotinamide and the dose of dopa or decarboxylase inhibitor in any of the groups of patients.

One intriguing problem is why clinical pellagra has not been reported in patients treated with dopa or dopa + a decarboxylase inhibitor; no physical signs indicative of pellagra were observed in any of the patients in this study. The excretion of N^1 -methylnicotinamide was low enough for it to be possible to classify most of these patients as niacin-depleted, if not with frank deficiency, and it has been shown previously that benserazide and carbidopa are considerably more potent inhibitors of kynurenine hydrolase than is isoniazid, which does cause clinical pellagra at similar doses (Bender & Smith, 1978). Isoniazid has a further action on niacin metabolism as it inhibits nicotinamide deamidase, the enzyme that converts

TABLE 2. Activity of nicotinamide deamidase from *Micrococcus lysodeikticus* in the presence of isoniazid, benserazide or carbidopa

Figures show mean values + SD, with the number of determinations in parentheses. * Significantly different from control ($P < 0.001$) by *t*-test.

Addition	Activity (nmol of nicotinic acid formed min ⁻¹ μg ⁻¹ of bacterial powder)
None	6.72 ± 0.96 (15)
Isoniazid (1 mmol/l)	3.55 ± 0.95 (5)*
Benserazide (16.7 mmol/l)	6.79 ± 0.79 (5)
Carbidopa (16.7 mmol/l)	6.33 ± 1.38 (5)

dietary nicotinamide into nicotinic acid (Johnson & Gadd, 1974). It is believed that only nicotinic acid, and not the amide, can act as a substrate for NAD synthesis, and most of the available dietary niacin is in the form of the amide rather than the free acid (Ijichi, Ichiyama & Hayaishi, 1966). Thus isoniazid prevents both endogenous synthesis of NAD from tryptophan and utilization of much of the dietary niacin, as well as re-utilization of nicotinamide released from NAD hydrolysis in the body. As can be seen from Table 2, neither benserazide nor carbidopa had any effect on the activity of nicotinamide deamidase from *M. lysodeikticus*, even at concentrations some 16-fold that of isoniazid to give 50% inhibition of the enzyme.

It therefore seems likely that the decarboxylase inhibitor-treated patients had a dietary intake of niacin which was adequate to meet their metabolic needs, and so to prevent the development of pellagra, despite inhibition of endogenous synthesis of NAD from tryptophan. This biochemical evidence of niacin depletion should alert clinicians to the possibility of pellagra in Parkinsonian patients who are receiving only marginally adequate diets.

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