Bensarazid with L-Dopa in the Treatment of Parkinson's Disease

A. VAN WIERINGEN

SUMMARY

A short review is given of the pharmokinetics and pharmacodynamics of the decarboxylase inhibitor Ro 4-4602. The results obtained in 20 patients using this drug in combination with L-dopa, (Madopar), are described.

Reduction in the total dosage of L-dopa by one-sixth to one-tenth the single preparation gave marked relief of nausea.

The induction period of the dosage was smoother, and an optimum dose could be reached sooner with earlier signs of improvement in comparison with the single drug.

S. Afr. Med. J., **48**, 206 (1974).

L-dopa (L-3,4-dihydroxyphenylalanine), an aromatic amino acid, although the present drug of choice in the treatment of patients with Parkinson's disease, is not an easy drug for use by inexpert hands. During the induction phase of treatment, its dosage has to be increased slowly, clinically titrated against the enzyme systems responsible for its degradation, adjusted to the patient's individual tolerance; and the maintenance phase is complicated by the appearance of side-effects.

In 30 patients treated with this drug, the author[25] encountered the same number of treatment failures and side-effects such as nausea and vomiting, postural hypotension, dyskinesias and psychic changes as described in other larger series. We have not seen cardiac arrhythmias as a complication.[24] Long-term treatment has shown that diurnal intermittent episodes of bradykinesia, so-called akinesia paradoxica[2] or the on-off phenomenon,[11] appear. It has been suggested that this may be related to the amount of protein in the diet.[10] An increase in serum growth hormone levels has been established, probably correcting a basic defective output of growth hormone in the disease itself,[16] but carrying a risk of iatrogenic acromegaly.[22]

This has led to the search for other substances which could modify the response of L-dopa, particularly those by which the total dose of L-dopa could be reduced.

DECARBOXYLASE INHIBITORS

Maximal degradation of orally-administered dopa probably occurs in the gastro-intestinal tract.[5] This is of the greatest importance because dopamine itself (Fig. 1) does not cross the blood-brain barrier, and the extent to which dopa is converted determines the quantity of dopa available for penetration into the brain. In man, absorption from the intestine is increased by the addition of a decarboxylase inhibitor.

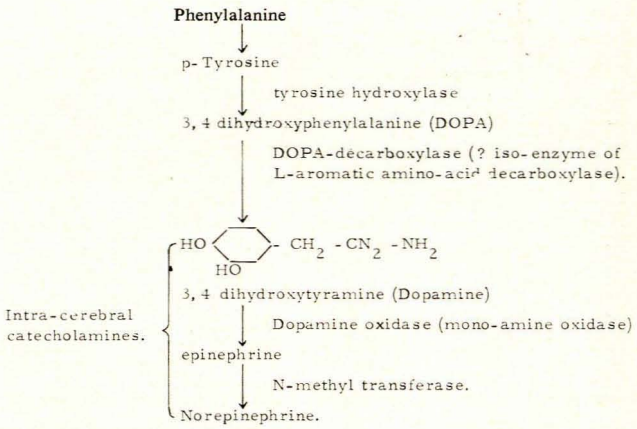

Fig. 1. Metabolic pathway involving dopa.

There are several substances with decarboxylase-inhibiting properties. These substances were developed more than a decade ago for the treatment of arterial hypertension, but it soon became clear that the decarboxylation of dopa was not a limiting step in the formation of catecholamines. In 1966 Udenfriend *et al.*[23] noted that a decarboxylase inhibitor (MK-485) actually decreased the dopa-induced increase of catecholamines in the brain *in vivo*. The following year the added beneficial effect of another decarboxylase inhibitor, bensarazid Ro 4-4602, to L-dopa in the treatment of Parkinson's disease, was described by Birkmayer and Mentasti.[6]

Ro 4-4602 is an amino-acid hydrazine derivative (*N*-(DL-seryl)-*N'*(2,3,4, trihydroxybenzyl)-hydrazine (HCl) (Fig. 2). In 1967 Bartholini *et al.*[4] demonstrated experimentally that the effect of Ro 4-4602 is due to preferential inhibition on extracerebral decarboxylase. This increased

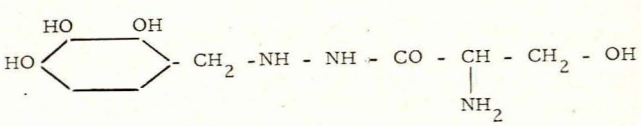

Fig. 2. Structural formula of bensarazid.

Department of Internal Medicine, University of Stellenbosch, and MRC Pigment Metabolism Unit, Bellville, CP
A. VAN WIERINGEN, M.B. CH.B., M.MED. (INT.), *Neurologist*

Date received: 28 August 1973

the titrated dopa in the blood, and consequently appeared to increase the supply of the brain with the amino acid.

Exogenous dopa is decarboxylated and further metabolised within the cerebral capillary walls as shown by histofluorometric methods;[12] the accumulated dopamine produces a green fluorescence of these structures. This dopa-induced green fluorescence disappears in the capillaries, and is enhanced in the brain parenchyma when Ro 4-4602 is given,[9] indicating that Ro 4-4602 inhibits the decarboxylase within the capillary walls, thereby protecting the dopa from being metabolised before entering the brain parenchyma.[18]

Experimentally Ro 4-4602 causes skeletal alterations in growing rats. This is of no clinical significance in patients with Parkinson's disease who have passed this developmental stage many years previously. In dogs, mass loss in combination with diarrhoea, fatty degeneration of the liver with impaired hepatic function (with protracted prothrombin time according to the Quick technique) and cytostatic effects on the bone marrow were noted. Terminal increase in serum urea was attributed to liver damage, as no changes in the kidneys could be detected.[19]

Clinical trials have demonstrated the effectiveness of Madopar.[3,7,14] Apart from gastric bleeding in patients who all had a previous history of peptic ulceration,[21] no toxic effects have been observed. However, all patients with such a history, patients suffering from systemic bone disease, patients with severe liver damage, and all female patients of child-bearing age, have been excluded from trials.

The main problem has been to establish the optimum ratio between dopa and Ro 4-4602. According to Barbeau[13] the safest clinical results (i.e. satisfactory improvement with minimal neurological side-effects) are obtained with and L-dopa : Ro 4-4602 ratio between 3 : 1 and 3 : 2, with a maximum of 400 mg daily of the decarboxylase inhibitor. Their previously recommended 4 : 1 ratio[1] gives optimal clinical effects on neurological signs, almost always causes severe hypotonia, and carries the risk of fairly abnormal involuntary movements.*

PATIENTS AND METHODS

In our trial the drugs supplied contained the ratios of L-dopa and Ro 4-4602 in the reverse sequence. From January 1972 until September 1972 the 3:2 combination was used: from September 1972 onwards the 4 : 1 ratio. The trial is thus divided into two sections.

Twelve patients were included in the trial on the 3:2 ratio of drugs. There were 6 men (aged 44 - 75 years) and 6 women (aged 55 - 70 years). The mean duration of their illness was 7½ years (ranging from 3 to 14 years). Ten were on L-dopa when treatment was started and their dosages varied from 2,5 g to 6 g per day. Their clinical disability was graded according to the stages of Hoehn and Yahr: 3 were in stage II; 5 in stage III; 4 were in stage IV. Changing over from L-dopa to Madopar caused no change in the clinical rating of their disability in 8 patients, 3 improved, and only 1 deteriorated. Apart from this single patient all preferred the combination drug, as this reduced the incidence of nausea very considerably. No change was noted in the occurrence of postural hypotension. One patient died during this period (female, aged 70 years, 14 years' duration of disease and clinically stage IV), from acute basilar artery thrombosis. No autopsy was done.

In the second stage of the trial on the 4 : 1 combination of drugs, 18 patients participated, including 10 patients from the previous group. These included 7 men (aged 44-75 years) and 11 women (aged 34 - 71 years). The mean duration of their illness was 9½ years (ranging from 1½ to 17 years). Of these, 4 were on L-dopa before starting treatment, 9 on the 3:2 combination and 5 newly-diagnosed patients. Their clinical disability varied from stage II (6 patients), stage III (8 patients) to stage IV (4 patients). Of the 5 patients starting on treatment for the first time, 2 could not tolerate the drug at all: one became violently nauseous after a single dose and the other very restless and agitated, so that in both the drug was immediately discontinued. The patient mentioned in the first group who discontinued use of the 3:2 combination, tried this 4:1 combination for 4 months without improvement and reverted to the L-dopa alone. The other 9 patients who were changed over from the 3:2 to the 4:1 combination showed no change in 6 patients and deterioration in 3. These 3 showed striking akinesia paradoxica. As it was felt that this was part of the long-term effect of L-dopa treatment, it could not be attributed to the combination of drugs; treatment was therefore not discontinued. The other 3 'new' patients all improved. Of 3 patients previously on L-dopa, 2 improved, and 1 showed no change. Again the most striking benefit obtained was relief of nausea.

Dosage

Where L-dopa had been used alone before, the dosage was first reduced by half, or more, until the signs and symptoms of Parkinson's disease became obvious again. A gradual substitution was then made with the new drug-combination until it was felt that the previous clinical state had been reached. Further dose increments were then made as required. However, at a later stage, an immediate switch-over from the single to the combination drug was made without any noticeable deleterious side-effects. Where a new patient started treatment the drug-combination was introduced slowly, with daily increases until a dose level was reached which was considered effective. In contrast to the single preparation, the introduction and increases in dose of the drug combination were smoother, accompanied by very little nausea, and a quicker response to treatment was seen. In the single drug this often took months to achieve, whereas the effect could now be seen within 1 - 2 weeks.

LABORATORY INVESTIGATIONS

During the first month of each trial laboratory investigations were carried out at 14-day intervals, thereafter at intervals of 6 - 8 weeks. These tests included erythrocyte

*Since this paper was submitted for publication, Barbeau has reaffirmed that the ratio best tolerated in practice is 4:1. (*Advances in Neurology* vol. 2, pp. 173 - 198. New York: Raven Press, 1973).

sedimentation rate (ESR), haemoglobin, haematocrit, red cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total white cell count and differential count, platelet count and reticulocyte count. Serum chemistry monitored included serum enzymes, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), lactic acid dehydrogenase (LDH), alkaline phosphatase (AP), total serum protein, serum albumin, serum globulin, total as well as fractionated serum bilirubin, uric acid, calcium, phosphate, urea and cholesterol levels.

Infrequent, scattered, transiently raised values of some of these enzymes were found from time to time in most patients, the one most frequently occurring being a raised serum LDH, which was found 18 times in 11 patients over a period of 18 months. One patient (male, aged 70 years, duration of illness 6 years, clinically disabled stage III, previously on L-dopa only at 3 g daily for 3 years) showed transient raised GOT, LDH and bilirubin values, never simultaneously, and each reverting to normal spontaneously without discontinuation of the drug. Only 1 patient showed fairly consistently raised urea values (female, aged 68 years, duration of disease 5 years, clinical degree of disablement stage III), but as she was a hypertensive of many years' standing, the raised urea was considered to be due to this condition and not to reflect a side-effect of the drugs. She also had a consistently raised ESR.

DISCUSSION

We have compared the amounts of L-dopa required when this drug is given singly or in combination with Ro 4-4602 (Table I). This comparison was possible in 13 patients; 7 patients had not been on the single preparation before and were therefore not included. The comparison shows that whereas the range of dosage of L-dopa on its own varied widely, from 1,5 g to 6 g, that of the 3:2 and 4:1 combinations varied from 375 mg to 800 mg and 300 mg to 800 mg respectively, with a mean of 502 mg and 545 mg respectively.

TABLE I. COMPARISON OF THE TOTAL DAILY DOSES REQUIRED OF L-DOPA ONLY AND OF THE L-DOPA CONTENT OF MADOPAR (3:2 and 4:1)

L-dopa (g/day)	3:2 combination L-dopa (mg/day)	4:1 combination L-dopa (mg/day)
1,5	—	300
2,5	450	600
3	450	600
3	450	—
3,5	450	400
3,5	450	600
4	375	—
4	800	300
4	450	600
4	—	800
5	450	600
6	600	600
6	450	600

There is therefore a very significant reduction in overall dosage of L-dopa. The narrower variation in dosage of the two drug combinations is due to the reduction in dose of L-dopa, correspondingly less nausea and vomiting, and those patients who could previously not tolerate an increase in L-dopa now approached dosages similar to those of patients who had been able to tolerate the higher doses.

At first sight, the difference in mean dosage of L-dopa between the two drug combinations appears small, giving the impression that in the final analysis there is little to choose between the two combinations. This is not so. With the 3:2 combination we quite quickly reached the maximum recommended 400 mg dose of decarboxylase inhibitor, and only exceeded this in 2 patients for a period of 6 weeks; in neither case was any added benefit obtained. With the 4:1 combination the maximum dose decarboxylase inhibitor was 200 mg, therefore permitting greater increase for the future if required. However, this increase could conceivably be offset by the disadvantageous effects recorded by Barbeau *et al.*[13]

The greatest advantage of the drug combination has been the very greatly reduced incidence of nausea and vomiting, suggesting that the site of this cause is pre-blood-brain barrier,[17,20] and related to the absolute dose of L-dopa.

There has been no observable effect on blood pressure.

Four patients have shown a steady deterioration throughout the 18 months of the trial. All were patients with marked symptoms of Parkinson's disease at the commencement of the trial, and the deterioration is presumably due to the natural course of the disease. Four patients have also shown a marked diurnal fluctuation in motor ability, mainly an afternoon increase in bradykinesia, a previously recognised long-term effect of L-dopa therapy.

Five patients have shown a decrease in the degree of involuntary movements, and 3 patients have remained the same. These involuntary movements are dose- and time-related, appearing in 50% of patients after 3 months, and in 80% after 2 years.[15] It would appear that the plasma concentration of L-dopa at which dyskinesias occur is an individual level for each patient, and that this level is the same on the single drug or the combination drug.[22] This suggests that attempts to improve therapeutic results by an absolute increase of the drug at the blood-brain barrier will be unsuccessful, except where the limiting factor previously has been severe gastro-intestinal side-effects.

The administration of L-dopa to experimental animals produces multiple biochemical changes.[5] One possible explanation for these involuntary movements is that decarboxylated L-dopa is distributed widely in the brain, while in adrenergic neurones only the enzyme dopamine hydroxylase reacts with dopamine to produce excess norepinephrine, which then mediates these movements. As dyskinesias are seldom seen in non-Parkinson patients treated with L-dopa, this suggests a denervation supersensitivity following extensive striatal degeneration.

Madopar (Ro 8-0576) (L-dopa + Ro 4-4602) was generously supplied by Messrs Roche Products.

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Standardisation of the Nitro-blue Tetrazolium Test and Factors Affecting its Clinical Application

R. SHER, R. ANDERSON, A. R. RABSON, H. J. KOORNHOF

SUMMARY

Attempting to standardise the nitro-blue tetrazolium (NBT) test, the following modifications of previously described procedures were introduced. Leucocyte-rich plasma obtained by simple gravitation at 37°C was mixed with NBT reagent which had previously been centrifuged to remove undissolved particles. Thick smears were made and stained with dilute haematoxylin, and physical manipulation during the various procedures was reduced to a minimum. Reconstitution of the buffy coat in fresh normal serum did not alter the NBT result.

Normal NBT readings were obtained in pregnant females, and women on contraceptive hormonal preparations.

S. Afr. Med. J., **48**, 209 (1974).

Department of Pathology (Microbiology), School of Pathology, South African Institute for Medical Research and University of the Witwatersrand, Johannesburg
SHER, M.B. B.CH.
ANDERSON, B.SC. HONS
R. RABSON, M.B. B.CH., D.C.P., D. PATH.
J. KOORNHOF, M.B. CH.B., D.C.P., DIP. BACT., M.R.C. PATH.

Date received: 27 August 1972.

The nitro-blue tetrazolium (NBT) test has been shown by a number of workers to be useful in the diagnosis of chronic granulomatous disease[1-5] and to monitor the progress of bacterial infections.[6] The test has also been shown to be positive in mycoplasma,[7] fungal, and protozoal infections such as malaria, and in various parasitic infestations.[8,9] The qualitative NBT test is now used as a routine in many hospital laboratories.[2,3,10,11]

Since the original description by Park *et al.*,[10] however, contradictory reports have appeared in the literature with regard to the normal NBT test values,[13,14] the effect of pregnancy on the results,[15] and the findings in viral meningo-encephalitis.[16] Because of the contradictions and the problems which were encountered in our laboratories, such as when neutropenia was present, and differentiating the formazan particle from heavily stained polymorphonuclear neutrophilic (PMN) nuclei using Romanowsky stain, the method of Park *et al.*[10] was critically examined. It was found necessary to introduce minor modifications mainly with a view to minimising undue stimulation of PMN cells during the laboratory procedures. Alterations to Park *et al.*'s[10] original method were also advocated by Freeman and King.[12,17] We are in agreement with most of their modifications. Our study was carried out indepen-