THE EFFECT OF LOW DOSE CARBIDOPA/LEVODOPA ON PROLACTIN AND GROWTH HORMONE CONCENTRATIONS IN PATIENTS WITH BREAST CANCER AND IN BENIGN BREAST TUMOURS

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- 1 Low doses of carbidopa/levodopa (12.5 mg 1- α -methyl-dopahydrazine, 125 mg levodopa) were administered orally to 29 patients with tumours of the breast (16 with breast cancer, 13 with benign breast disease).
- 2 Plasma dopa response curves were similar in all the patients studied.
- 3 Prolactin and growth hormone showed similar responses to carbidopa/levodopa irrespective of age or diagnosis.
- 4 Prolactin showed an unusual response in four patients which has not previously been recorded.

Introduction

Levodopa is a dihydroxyphenylalanine which is thought to pass the blood brain barrier and undergo transformation to dopamine in the brain (Hornykiewicz, 1966; Lloyd & Hornykiewicz, 1970). Dopamine blocks midbrain catecholamine synthesis and suppresses prolactin secretion either by facilitation of the release of the hypothalamic substance prolactin inhibiting factor (PIF) (Kamberi, Mical & Porter, 1971) or by direct action on the pituitary gland (Diefenbach, Carmel, Frantz & Ferin, 1976), or both (McCann, Kabra, Donoso, 1972). Boyd, Lebowitz & Pfeiffer (1970) found that levodopa raises serum growth hormone (GH) probably by acting upon hypothalamic α -adrenergic receptors and stimulating the release of human growth hormone releasing factor.

Although prolactin is suspected of being a major factor in carcinogenesis in the human breast (Stoll, 1969), much of the evidence suggests that, in patients with breast cancer, serum prolactin concentrations are within the normal range compared with those of control groups (Boyns, Cole, Griffiths, Roberts, Buchan, Wilson & Forrest, 1973; Franks, Ralphs, Seagroatt & Jacobs, 1974; Mittra, Hayward & McNeilly, 1974). However, abnormal values have been found in unaffected members of families with a high prevalence of breast cancer (Kwa, De Jong-Bakker, Engelsman & Cleton, 1974).

The results of single specimen analyses must be viewed with caution as it is difficult to detect abnormalities in prolactin homeostasis by these

means. We decided, therefore, to study the response of prolactin to the administration of low doses of carbidopa/levodopa in breast cancer patients and in patients with benign breast disease. We also studied growth hormone responses in the same groups.

Methods

Twenty-nine patients, 16 with breast cancer and 13 with benign breast disease, were studied after informed consent had been obtained. The diagnosis had been confirmed histologically following operations performed between two and four years previously. Only patients with breast cancer Stage I or II of the Manchester Classification (Wise, York Mason & Ackerman, 1971) were included in the cancer group. None of the patients had been on medication during the week preceding the test apart from one patient who was taking thyroxine. The ages of the patients with benign breast disease ranged from 33–68 years (mean 48) and those with cancer from 46–69 years (mean 57).

After an overnight fast the test procedure began at 09.00 h with the patient lying on a bed. A catheter was inserted into an antecubital vein and was kept patent by means of a 3.8% solution of sodium citrate. Blood was taken at 20 min and at zero time, then the patient was given Sinemet (125 mg levodopa and 12.5 mg 1- α -methyl-dopahydrazine; Merck, Sharpe and Dohme) by mouth. Blood samples were taken at 30, 60 and 90

min and at 2,3,4 and 6 h. The initial 2 ml of each blood sample was discarded. The plasma of the remaining sample was separated in a centrifuge at 1000 rev/min for 10 min and immediately stored at -20°C .

Prolactin was measured by a specific double antibody radioimmunoassay using an antihuman prolactin antiserum raised in rabbits; purified human pituitary prolactin was used as standard and for iodination (McNeilly, 1973). The sensitivity of the assay was 80 $\mu\text{u/l}$, inter-assay and within-assay variation less than 10%.

Human growth hormone was measured by a modification of a radioimmunoassay employing an antiserum raised in a rabbit by immunization with purified human growth hormone. MRC standard HGH (66/217) was used for standardization and iodination. Separation was by double antibody technique (Kirkham & Hunter, 1971).

Dopa was determined using oxidation by ferricyanide after precipitation of protein and elution

from Dowex 50. A second sample of the eluate was heated at pH 8.5 to destroy dopa and 3-*O*-methyldopa determined using oxidation by iodine (Curzon, Bharati, Kantamaneni & Trigwell, 1972).

The concentrations of prolactin, human growth hormone and dopa were charted against time.

Results

The plasma concentrations of dopa and prolactin following administration of 125 mg of carbidopa/levodopa are shown in Tables I and 2. The shape of the plasma dopa curves varied only slightly from patient to patient with peak plasma dopa levels occurring at 90 min (range 30-120 min) the mean peak plasma concentration being 1025 nmol/l (range 200-1980 nmol/l). Analysis with Student's *t*-test showed that there was no significant difference between the groups. The plasma dopa concentrations did not show a statistically significant correlation with age. Nausea (with vomiting in one patient) was the

Table 1 Plasma dopa concentrations (n mol/l) following administration of carbidopa/levodopa

Women with breast cancer

Patient number	Time (min)						
	30	60	90	120	180	240	360
Plasma dopa concentration (nmol/l)							
1	100	405	1120	510	200	100	0
2	100	405	1120	1015	305	200	100
3	150	355	1120	510	305	150	100
4	150	455	1015	760	100	0	0
5	200	610	1980	1675	760	405	200
6	0	355	1120	760	255	255	100
7	50	455	1520	1675	810	355	255
8	150	405	710	1015	405	200	200
9	200	510	610	100	255	0	0
10	255	710	760	1015	405	355	100
11	200	455	1015	1520	1065	610	200
12	200	100	150	100	200	100	0
13	100	355	710	510	150	0	0

Women with benign breast tumours

Patient number	Time (min)						
	30	60	90	120	180	240	360
Plasma dopa concentrations (nmol/l)							
1	0	455	1520	1625	405	305	150
2	100	405	1015	355	405	305	100
3	100	355	1120	1520	405	200	100
4	150	405	1015	1015	355	100	0
5	150	405	1520	1625	455	405	150
6	200	610	1675	1625	455	255	0
7	255	255	610	200	235	100	0
8	455	510	1625	1675	510	510	405
9	255	510	1120	1520	610	560	255
10	200	255	1120	1015	355	150	0
11	0	455	1520	1520	405	200	100
12	50	355	1120	1015	305	150	0

only consistent side effect, but did not seem to be related to peak plasma concentrations.

Basal plasma growth hormone concentrations were consistently low. Only eight patients showed an unequivocal rise following carbidopa/levodopa as evidenced by at least a 12 mU/l rise in the plasma level, the peak increment generally occurring at 120 min, 30 min after the plasma dopa peak. Those who responded were representative of the group as a whole in terms of plasma dopa concentrations (355–1520 n mol/l), and diagnosis (five breast cancer, three benign breast disease).

Analysis of the results using the Student's *t*-test showed that basal plasma prolactin concentrations were not significantly different in the group with breast cancer from the group with benign breast disease. Although evaluation of suppression in the groups studied is obscured by the sensitivity of the assay (80 mU/l), the similarity in response between the two groups is evident. Prolactin was suppressed in all patients studied, nadir values occurring generally at 120 min. Four patients showed an unusual prolactin

response and their results are not included in the tabulated values. In these four patients we repeated the experiment under the same conditions 3 months later and the response was repeatable in only one patient who was under treatment with thyroxine (Figure 1); the plasma dopa concentrations being similar to those when originally tested. Figure 1 is typical of the initial response seen in the four patients, their prolactin concentrations reached approximately the same high values. In this group prolactin levels were suppressed to below 80 mU/l at 2 h, then rose to between 2–3000 mU/l at 4 h, at 6 h the concentrations were falling (normal range in healthy women without breast disease 80–360 mU/l).

Discussion

The basal plasma prolactin concentrations in the patients with early breast cancer were not significantly different from those of patients with benign breast disease and lay within the normal range. These results

Table 2 Effect of carbidopa/levodopa on plasma prolactin (mU/l)

Women with breast cancer

Patient number	Time (min)								
	-20	0	30	60	90	120	180	240	360
Plasma prolactin (mU/l)									
1	140	100	140	140	120	<80	100	240	280
2	300	140	120	120	<80	<80	<80	100	140
3	120	120	120	120	<80	<80	<80	120	100
4	120	80	100	80	80	<80	<80	100	160
5	260	260	280	300	240	80	240	260	340
6	260	260	160	160	<80	<80	140	100	100
7	140	140	100	100	<80	<80	80	160	100
8	100	160	160	160	<80	<80	<80	80	120
9	300	200	120	120	<80	<80	<80	280	120
10	180	120	100	120	<80	<80	80	220	120
11	120	120	100	180	<80	<80	<80	160	120
12	100	100	100	80	80	<80	<80	100	140
13	120	120	120	120	<80	<80	<80	120	220

Women with benign breast tumours

Patient number	Time (min)								
	-20	0	30	60	90	120	180	240	360
Plasma prolactin (mU/l)									
1	120	120	80	80	80	<80	<80	120	180
2	220	280	120	120	120	<80	200	140	160
3	100	160	100	100	100	<80	<80	80	200
4	160	120	120	120	<80	<80	<80	80	220
5	100	100	160	100	100	<80	80	140	220
6	220	200	180	100	80	<80	<80	100	160
7	260	200	340	100	260	80	260	320	200
8	280	240	120	80	<80	80	100	160	200
9	220	260	300	360	240	80	160	240	240
10	320	200	120	100	80	80	<80	80	180
11	220	280	300	180	100	80	100	160	260
12	220	240	220	180	160	80	240	240	160

Figure 1 The result for one of the four patients who showed an unusual prolactin response. This patient was receiving L-thyroxine (0.3 mg/day). The horizontal dotted line indicates the minimum level of sensitivity of the method.

are in agreement with those of other workers (Boyns *et al.*, 1973; Franks *et al.*, 1974; Mittra *et al.*, 1974).

The plasma levodopa response curves are similar to those previously published (Mars & Genuth, 1973; Tyce, Muentner & Owen, 1970), though there was less inter-patient variation in the levodopa concentrations. The lowest concentrations of prolactin and the peak concentrations of growth hormone (in those patients who showed a definite response) followed the peak plasma dopa concentrations by 30 min, which suggests that it takes this time for levodopa to penetrate the central nervous system and undergo decarboxylation to the active constituent, dopamine.

There is a significant correlation between the dose of levodopa administered and the plasma dopa concentration achieved (Tyce *et al.*, 1970). In normal subjects it has been reported that oral doses of 250–500 mg levodopa suppress prolactin secretion (Kleinberg, Noel & Frantz, 1971). Mars & Genuth (1973) suggested that a minimal levodopa concentration of 2030 n mol/l was necessary to evoke a rise in growth hormone. In all our patients prolactin was suppressed with 125 mg levodopa administered orally,

which produced peak plasma dopa concentrations of 1025 n mol/l (range 200–1980 n mol/l). In eight patients stimulation of growth hormone secretion was produced by a dopa concentration range of 355–1420 n mol/l.

There were no differences in either the suppression of prolactin or the stimulation of growth hormone production between the patients with breast cancer and those with benign disease. There is evidence that hypothalamic sensitivity to stimulation or inhibition tends to rise with age and it has been postulated that, in patients with cancer, premature ageing of the hypothalamus may occur (Dilman, 1974). However, we found no age related differences, in growth hormone or prolactin responses to levodopa in either the breast cancer group or in the patients with benign disease.

Levodopa normally produces a decrease in blood prolactin concentration followed by a small rebound at about 8 h (Rozenzweig, Heusen, Bila, L'Hermite & Robyn, 1973). However, in four of our 29 patients we observed an excessive rebound secretion of prolactin, with concentrations up to ten times the normal. Three patients had breast cancer and one, with benign breast disease, was receiving 0.3 mg of L-thyroxine per day for hypothyroidism. This enhanced secretion of prolactin following levodopa suppression has not been reported before. It suggests that there may be two types of dopamine receptor, one excitatory and the other inhibitory. Behavioural studies in rats (Cools, Janssen, Struyker Boudier & Van Rossum, 1975) and in the rhesus monkey (Cools, Hendriks & Korten, 1975) support this concept.

This abnormal response was only repeatable in the one patient who was receiving L-thyroxine (Figure 1). This may be explained by the fact that we were using low doses of levodopa and one might expect to get variable distributions in the central nervous system in the same patient on different occasions.

If abnormal surges of prolactin do occur in breast cancer patients, the phenomenon may be of importance in the aetiology and progression of breast cancer, since one fifth of breast cancers are dependent on prolactin for growth (Hobbs, De Souza, Salih & Raggatt, 1974). It has been shown that there is a higher prevalence of breast cancer in patients with hypothyroidism in whom prolactin concentrations may also be elevated (Mittra *et al.*, 1974). Further work on the mechanisms of secretion of prolactin in patients with breast cancer is being carried out.

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