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REDUCED INTRAVENOUS GLUTATHIONE IN THE TREATMENT OF EARLY PARKINSON'S DISEASE

GIANPIETRO SECHI[1], MARIA G. DELEDDA[2], GUIDO BUA[1], WANDA M.SATTA[1], GIOVANNI A. DEIANA[1], GIOVANNI M. PES[3] and GIULIO ROSATI[1]

[1]Department of Neurology, [2]Division of Internal Medicine, and [3]Chair of Clinical Biochemistry, University of Sassari, Sassari, Italy.

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Abstract

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1. Several studies have demonstrated a deficiency in reduced glutathione (GSH) in the nigra of patients with Parkinson's Disease (PD). In particular, the magnitude of reduction in GSH seems to parallel the severity of the disease. This finding may indicate a means by which the nigra cells could be therapeutically supported.
2. The authors studied the effects of GSH in nine patients with early, untreated PD. GSH was administered intravenous, 600 mg twice daily, for 30 days, in an open label fashion. Then, the drug was discontinued and a follow-up examination carried-out at 1-month interval for 2-4 months. Thereafter, the patients were treated with carbidopa-levodopa.
3. The clinical disability was assessed by using two different rating scale and the Webster Step-Second Test at baseline and at 1-month interval for 4-6 months. All patients improved significantly after GSH therapy, with a 42% decline in disability. Once GSH was stopped the therapeutic effect lasted for 2-4 months.
4. Our data indicate that in untreated PD patients GSH has symptomatic efficacy and possibly retards the progression of the disease.

Keywords: Parkinson's Disease; reduced glutathione.

Abbreviations: Columbia University Rating Scale (CURS); Parkinson's Disease (PD); Patients Global Impressions (PGI); reduced glutathione (GSH); resting tremor (RT); Webster Step-Second Test (W.S.S.T.).

Introduction

The mechanisms underlying dopamine cells death in the zona compacta of substantia nigra in Parkinson's disease (PD) remain unclear. However, current concepts of this process indicate that free radicals generated by oxidation reactions may play a key role (Jenner et al., 1992). Indeed, in postmortem tissues from patients with PD there is evidence for inhibition of complex 1 of the mitochondrial respiratory chain, altered iron metabolism and decreased levels of reduced glutathione (GSH) (Riederer et al., 1989, Jenner, 1993). Of these defence mechanisms implicated in the prevention of free-radical-induced tissue damage, only the reduction in the levels of GSH in substantia nigra appears to be specific to PD (Jenner, 1993, Sian et al., 1994) and, noteworthy, this reduction has been also found in cases of incidental Lewy body disease (presymptomatic PD) (Perry et al., 1982, Sian et al., 1992). In particular, the magnitude of reduction in GSH seems to parallel the severity of PD and, in advanced stages, in the nigra, GSH is virtually undetectable (Riederer et al., 1989). In addition, data from animal studies have shown that an induced GSH depletion in mice produces morphological changes in nigral dopamine neurons resembling those seen in normal aging and in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxicity (McNeil et al., 1986). These observations have led us to determine the effect of intravenous (i.v.) GSH in patients with early, untreated PD.

Methods

Patients

After giving informed consent, 9 consecutive patients with idiopathic PD were enrolled in the study. There were 6 men and 3 women, age 66 ± 9 years (mean ± SD) (range, 49 to 77); Hoehn and Yahr stage of parkinsonism 2 ± 0.9 (mean ± SD) (range 1 to 4), with a disability duration of 13 ± 4.5 months (mean ± SD) (range, 8 to 24). The patients were considered eligible for the study, provided that they had not been treated previously with any antiparkinsonian drug or other agents active on the central nervous system including deprenyl or vitamin E. The patients with dementia (Mini-Mental State Examination), or depression (Hamilton Rating Scale) were excluded.

Procedures

GSH was administered i.v. 600 mg in 250 ml saline, as 1-hour infusion, twice daily, at 8.00 A.M. and 4.00 P.M, for 30 days, in an open label fashion. The patients were assessed at baseline and 30 days after the treatment. Then, GSH

was discontinued and, if the patient status improved, a follow-up examination was carried-out at 1-month interval, until the patient's clinical status returned to baseline, or when the patient felt he or she was worsened. Thereafter, the patients were treated with carbidopa-levodopa (25-250 mg), half-tablet three times daily, and a new examination carried-out after 30 days, about two hours after the intake of the drug.

Assessments

On each visit, the clinical disability was assessed according to a modified Columbia University Rating Scale (CURS) (Yahr et al., 1969), and to the Webster Step-Second Test (W.S.S.T.) (scoring method: time in seconds to stand and walk a prescribed course and sit again) (Webster, 1968).

The subscores evaluated at the modified CURS were: speech, hypomimia, tremor at rest, action or postural tremor of hands, rigidity, finger taps, hand movements, pronation and supination of hands, foot taping, arising from chair, posture, gait, balance and hypokinesia.

For the W.S.S.T., three sequential trials were performed for each patient, at baseline and at each control, with a fixed intertrial interval of 15 s. In the tabulation of the results the authors used the mean (± SD) of the three W.S.S.T. values obtained, for each patient, at the beginning of the experiment and after each of the trial periods.

Clinical response was also self assessed by patients according to Patients Global Impressions (PGI) (Guy, 1976). This scale (ranging from 1 = very much better, to 7 = very much worse) was used to assess the change in severity of the disease from the beginning of the study and from the previous visit.

All patients were evaluated by the same examiner throughout the study. On each examination, they were observed over two consecutive days. In addition, in patients with tremor, at baseline and at each examination, at approximately the same time of day, tremograms were recorded using an accelerometer transducer attached to the index finger of the hand and recorded on an EEG polygraph. Tremor frequency (in Hertz) and visual mean amplitude (mean value of tremor estimated visually in microvolts) were measured from the tracings.

Laboratory Assessments

The following laboratory tests were performed at entry and after 30 days of GSH therapy: routine blood chemistries, liver function tests, blood counts, urinalysis and ECG. A chest X-ray and a brain CT, performed without contrast, were conducted in all patients before study entry.

Data Analysis

A statistical analysis of W.S.S.T. values was made, for each patient, by Student's *t* test for paired samples. A nonparametric statistical method was used to compare clinical parkinsonian scores (Wilcoxon Matched-pairs Signed-ranks Test) and the PGI (McNemar's Test). In addition, the sums of clinical parkinsonian scores for each patient, at baseline, were correlated either with the percent improvement calculated through the same scale, or with the percent improvement at W.S.S.T. after GSH therapy. The percentage of change was calculated by the following formula:
prestudy value - treatment value / prestudy value x 100 = % change. The level of significance was $p < 0.05$.

Results

All 9 patients enrolled completed the study. There were no serious complications from i.v infusion of GSH. Two patients during the third week of i.v GSH treatment had fever (axillary, peak temperature, 38.2°C), erithemia of the skin, irritation and hardness at injection site, likely due to infusion thrombophlebitis. The irritation and fever cleared up after 5 days of antibiotic and antiphlogistic therapy. Patient 6 after completion of the wash-out period suffered a thigh-bone fracture. GSH did not induce clinically significant changes in any laboratory test compared with basal conditions. The brain CTs showed a mild cortical atrophy in two patients and no definite abnormalities in seven of them. The frequency of resting tremor (RT) was 5 to 6 Hz. In patient 1, the mean amplitude of RT, compared with the baseline period, was reduced, approximately, by 50% after GSH therapy, and by 25% after carbidopa-levodopa (Fig 1). No definite variations in the mean amplitude of RT were noted in the other patients, in the various sequences of treatment, with respect to baseline, or to the wash-out period. At W.S.S.T. all patients improved significantly after GSH, with respect to baseline (from $p < 0.05$, to $p < 0.01$); instead, after levodopa-carbidopa, only five patients improved significantly, with respect to the wash-out period (from $p < 0.02$, to $p < 0.01$). (Table 1). In our opinion, for the dosages of levodopa-carbidopa and GSH used, the transient improvement induced by these drugs was roughly comparable. The total scores for parkinsonian disability (modified CURS) were significantly lower either after GSH therapy, with respect to baseline ($p < 0.007$), or after levodopa-carbidopa with respect to the wash-out period ($p < 0.01$). (Table 2). A significant improvement after GSH therapy, with respect to baseline, of modified CURS subscores, was evidenced for speech, hypomimia, rigidity,

pronation and supination of hands, foot taping, posture, gait, balance and hypokinesia (from $p < 0.02$, to $p < 0.007$).

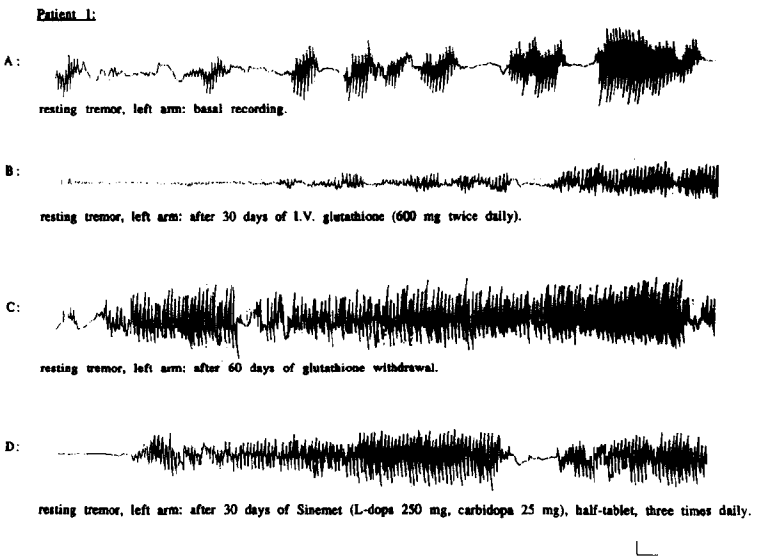

Fig 1. Representative resting tremor recordings in patient 1, at baseline (A); 30 days after therapy with reduced glutathione (B); 60 days after withdrawal of reduced glutathione (C); and 30 days after carbidopa-levodopa (D): Vertical calibration is 200 microvolts; horizontal scale is 1 second.

A similar improvement was noted after carbidopa-levodopa, with respect to the wash-out period (from $p < 0.04$, to $p < 0.01$). As seen from Tables, the values of the modified CURS scores and the values of W.S.S.T., after withdrawal of GSH therapy, reached the baseline values after 2.6 ± 0.7 months (range, 2 to 4 months). The correlation coefficient between total CURS scores at baseline, and the percent improvement calculated through the same scale, after GSH therapy, is shown in Fig 2. In Fig 3, is shown the correlation coefficient between total CURS scores at baseline and the percent improvement at the W.S.S.T. after GSH therapy. The slope of this late correlation is significantly different than zero ($r = 0.6813$; $p = 0.0433$), while the correlation shown in Fig 2 is non-significant.

Table 1

Webster Step-Second Test (W.S.S.T): Scoring Method: Time in Seconds to Stand and Walk a Prescribed Course and Sit Again (mean ± SD, of 3 sequential trials).

Patient	W.S.S.T. At Baseline	W.S.S.T. After GSH	% Improv.$_1$	Wash-out (months)	W.S.S.T. After Wash-out	W.S.S.T. After Lev.+DCI	% Improv.$_2$
1	46.8 ± 1.6	41.2 ± 0.3**	12	3	45.5 ± 0.86	43.0 ± 0.5**	5.5
2	58.7 ± 0.6	57.2 ± 0.6*	3	4	65.7 ± 1.1	63.7 ± 1.1	3
3	34.0 ± 0.1	33.0 ± 0.5*	3	3	37.7 ± 0.58	34.7 ± 0.57**	8
4	34.3 ± 0.5	33.3 ± 0.3*	3	2	42.7 ± 0.58	42.0 ± 0.5	2
5	59.0 ± 1.8	52.0 ± 1.8**	12	2	51.2 ± 0.3	50.0 ± 0.1**	2.5
6	32.9 ± 4.3	25.6 ± 1.5*	22	2	32.3 ± 2.6	-	-
7	44.3 ± 1.15	39.7 ± 0.58**	10	2	45.0 ± 1.2	39.6 ± 1.9***	12
8	51.7 ± 0.8	49.4 ± 1.0*	4.5	2	52.0 ± 1.2	48.2 ± 1.3***	7
9	25.0 ± 0.1	22.7 ± 1.1*	9	3	24.3 ± 0.6	24.0 ± 0.1	1

Lev.+DCI=Levodopa+Decarboxylase Inhibitor; W.S.S.T. values after GSH were compared with baseline; W.S.S.T. values after Lev.+DCI were compared with the wash-out period; *$p<0.05$; **$p<0.01$; ***$p<0.02$ (Student's t Test for paired samples).

Table 2

Modified Columbia University Rating Scale: Total Scores in 9 Patients with Parkinson's Disease Treated with GSH (600 mg/day, I.V.) or Levodopa (375 mg/day + DCI, per os).

Patient	Baseline T. Scores	T. Scores After GSH	% Improv.$_1$	Wash-out (months)	T. Scores After Wash-out	T. Scores After Lev.+DCI	% Improv.$_2$
1	25	15	40	3	24	14	42
2	12	3	75	4	16	3	81
3	29	20	31	3	27	20	26
4	15	7	53	2	18	7	61
5	35	17	51	2	30	14	53
6	42	26	38	2	42	-	-
7	20	15	25	2	21	14	33
8	31	19	39	2	32	21	34
9	34	25	27	3	35	26	26
Mean ± SD	27 ±10	16 ± 8*	42 ± 16	2.6 ± 0.7	27 ± 8	15 ± 7**	44.5 ± 19

Lev.+DCI=Levodopa+Decarboxylase Inhibitor; T. Scores=Total Scores; T. Scores values after GSH were compared with baseline; T. Scores values after Lev.+DCI were compared with the wash-out period; *$p<0.007$; **$p<0.01$; (Wilcoxon Matched-pair Signed-ranks Test).

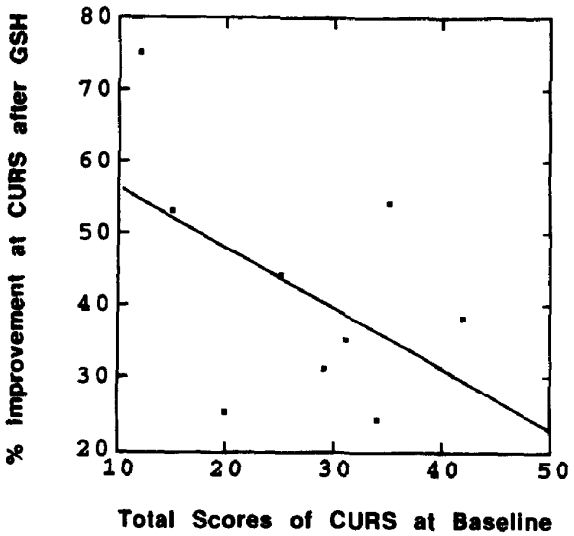

Fig 2. Correlation coefficient between total CURS scores at baseline and the percent improvement calculated through the same scale after therapy with reduced glutathione (r= - 0.5027; p=n.s.).

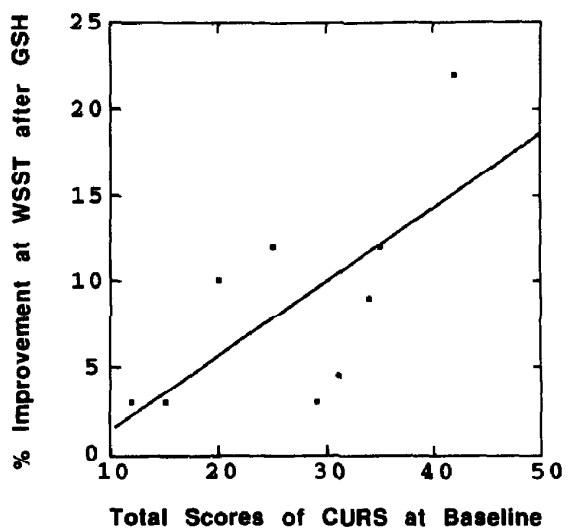

Fig 3. Correlation coefficient between total CURS scores at baseline and the percent improvement at the W.S.S.T. after therapy with reduced glutathione (r=0.6813; p=0.0433).

Total scores of PGI were significantly lower either after GSH therapy, with respect to wash-out period (or, baseline) ($p < 0.0039$) or after levodopa-carbidopa, with respect to the wash-out period ($p < 0.0039$), where, no significant difference was found at PGI between GSH therapy and levodopa-carbidopa. One patient (n.9) with a marked sialorrhea, reported the disappearance of this symptom after GSH therapy. After withdrawal of GSH the benefit lasted for about 3 months. Levodopa-carbidopa therapy was ineffective on sialorrhea in the same patient.

Discussion

The crucial observation, that in idiopathic PD the magnitude of reduction in GSH in substantia nigra seems to parellel the severity of the disease, may indicate a means by which the nigra cells could be therapeutically supported (Riederer et al., 1989).

GSH and the Blood-Brain Barrier

GSH is a tripeptide (gamma-glutamyl-cysteinyl-glycine) which in physiological conditions is believed to be extracted in minimal amount at the blood-brain barrier (Cornford et al., 1978). In addition, as it is a naturally occurring peptide, the possibility exists that there may be a breakdown of glutathione in plasma by peptidases, as in other tissues and at blood-brain barrier itself (Meister and Tate, 1976). Therefore, its clinical value as a therapeutic agent, if administered by a peripheral route, should be minimal. However, since recent investigations support the concept of a selective transcytosis for many peptides across an intact blood-brain barrier (Pardridge, 1986), and since the finding that in idiopathic PD the locus coeruleus, which helps to preserve the integrity of blood-brain barrier functions, is damaged (Tomonaga, 1983, Harik and McGunigal, 1984), the authors administered GSH as 1-hour infusion two times daily for 30 days in PD patients, to investigate a possible therapeutic effect of this peptide, after peripheral administration. Actually, recent experimental evidences have shown blood-brain extraction of circulating GSH in a brain perfusion model, and the transcytosis of intact GSH into the brain parenchyma without breakdown (Zlokovic et al., 1994).

Effects of Intravenous GSH in Parkinson's Disease

The results of our open study indicate that in PD this peptide given i.v. may reach its specific target in the brain (i.e, the nigra cells) and may have a significant beneficial effect on several parkinsonian signs. In particular, as

shown in Fig 3, the therapeutic effect of GSH on hypokinesia appears to be correlated to the severity of the symptom. This peptide was also effective in reducing the RT in one patient, but failed in other four. In this patient, GSH apparently improved the RT more than levodopa-carbidopa. In our opinion, since the dosages of levodopa-carbidopa and GSH used are not comparable, to draw this conclusion is incorrect. Once GSH was stopped the therapeutic effect lasted for 2-4 months. This finding, in our opinion, is a strong evidence against a placebo effect and this may indicate a protective effect of the drug on the rate of progression of PD. However, this does not necessarily exclude a symptomatic effect of GSH. These concepts are supported by the results of two double-blind studies on the use of selegine, or bromocriptine versus placebo in PD (Teychenne et al., 1982, Myllylä et al., 1992). Indeed, in these studies, the mean CURS scores in the placebo group returned to baseline after about 1-month, and the symptomatic effect of bromocriptine, once stopped, did not last for more than four weeks (Teychenne et al., 1982, Myllylä et al., 1992).

Hypothetical Mechanisms Underlying the Therapeutic Effect of GSH

The mechanism underlying the therapeutic effect of GSH in PD is unknown. According to the most basic neurochemical abnormality in the brain of PD patients (i.e., the marked loss of dopamine in the nigrostriatal neuron system) (Ehringer and Hornykiewiez, 1960), an action of GSH at dopaminergic synapses (presynaptically or postsynaptically) can be hypothesized. In particular, based on the theoretical notion that decreasing the oxidative load in substantia nigra may slow disease progression, it would seem that GSH, because of its antioxidant properties (e.g., reduced formation of hydrogen peroxide), may be able to protect the striatonigral cells and foster dopaminergic activity (Jenner, 1993). Recent evidences of glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes fit this hypothesis (Volterra et al., 1994).
Another important biological function that has been ascribed to glutathione is the role in translocation of amino acids, and possibly also peptide, across cell membranes (Meister and Tate, 1976). This function may be important for the transport of substrate and specific proteic neurotrophic factors into the dopaminergic neurons of the substantia nigra (Tooyama et al., 1993). Given the reduction in the levels of GSH in cells of substantia nigra, in PD this function is likely impaired. A replacement therapy with exogenous GSH may contribute to its reinstatement. A controlled study of this peptide for the treatment of PD seems warranted.

Conclusion

The findings indicate that in PD GSH given i.v. may reach its specific target in the brain (i.e., the nigra cells), it has a significant beneficial effect on several parkinsonian signs and possibly retards the progression of the disease.

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Inquiries and reprint requests should be addressed to:

GianPietro Sechi, M.D.
Neurological Clinic
Viale S. Pietro, 10
07100-Sassari
Italy.