J. Pharm. Pharmacol. 1994, 46: 515–517
Received September 2, 1993
Accepted December 15, 1993

The effect of theophylline on parkinsonian symptoms

JUDIT MALLY, T. W. STONE*, *Department of Neurology, Central Hospital Fejer, Szekesfehervar, Jozsef 8, Hungary H-8000, *Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, UK*

Abstract—Adenosine is known to inhibit the release of dopamine from central synaptic terminals. The present open trial was therefore conducted to determine whether the adenosine receptor-antagonist theophylline would be of value in Parkinson's disease. Fifteen parkinsonian patients were treated for up to 12 weeks with a slow release oral theophylline preparation (150 mg day^{-1}), yielding serum theophylline levels of 4·44 mg L^{-1} after one week. The patients exhibited significant improvements in mean objective disability scores and 11 reported moderate or marked subjective improvement. It is suggested that theophylline might be a useful adjunct to the routine therapy of parkinsonian patients.

The main aetiological factor in Parkinson's disease is the depletion of dopamine in the striatum which is the consequence of degeneration of the substantia nigra. Any modulation of dopamine release in striatum may therefore influence the symptoms of Parkinson's disease. Agonists at adenosine A_2 receptors are known to reduce locomotor activity and it has been suggested recently that this is due to the A_{2a} receptors located in the striatum, causing a decreased release of dopamine (Barraco et al 1993). Furthermore the A_{2a} adenosine receptors decrease the affinity of dopaminergic ligands for postsynaptic D_2 receptors (Ferre et al 1991).

In view of the above observations it was hypothesized that antagonizing A_{2a} adenosine receptors with the xanthine antagonist theophylline might increase the release of dopamine and consequently improve parkinsonian symptoms. This hypothesis was tested in a short open study.

Materials and methods

Fifteen outpatients with idiopathic Parkinson's disease were included in this study. The protocol of the trial was approved by the local ethical committee and written informed consent was given by the participants.

The selection criteria were that the patients with Parkinson's disease had been stable for at least three months before the trial. All were being controlled with levodopa and carbidopa combined treatment, but none showed signs of on-off effects. No improvement of the parkinsonian symptoms could be achieved by further elevation of the dose of levodopa because of adverse reactions including drowsiness, dizziness and weakness. In this study, 10 males and 5 females were followed for three months. They were between 60 and 80 years (average 67 ± 2 yr). Of the 15 patients, 7 were categorized as Hoehn-Yahr stage II and 8 as Hoehn-Yahr stage III (Hoehn & Yahr 1967). The existing duration of the disease was between 3 and 13 years (average 6 ± 1). Eleven patients reported hand tremor and 4 hypokinesis as a first sign of the disease. All of the patients began therapy with 10 mg day^{-1} selegiline (Jumex), which was maintained throughout the trial, and levodopa (Madopar) was added gradually. The dose range needed in this group of patients was between 60 and 750 mg day^{-1} (average: 355 ± 75 mg day^{-1}). These are submaximal doses but increasing the dose increased the severity of side-effects including nausea, vomiting, dizziness and drowsiness to such an extent that the participants began to refuse the drugs. This therapy was maintained for three months before commencing the theophylline trial. The last three parkinsonian scores assessed before the study were taken as control data. Slow release theophylline (Theophtard Biogal RT, Hungary) was given once a day orally. After the introduction of theophylline the patients were examined once a week in the morning hours after taking their drugs.

The parkinsonian features were assessed by two scores. One of them was a graded clinical rating scale (Mally 1992) which includes an assessment of rigidity and walking ability. Normal function was given as 0, the total disability was 66 points. The other measure used was the Unified Parkinson's Disease Rating Scale (UPDRS) which is based upon a series of assessments of subjective mental, motor and daily living activities including tremor and hypokinesia (Lang & Fahn 1989). Patients' subjective opinions about the introduced theophylline therapy were invited in the following categories: worse, no change, moderate improvement, marked improvement.

The concentration of theophylline in serum was measured by radioimmunoassay at the time of the physical examination.

For statistical analysis, an unpaired Student's *t*-test and Kruskall–Wallis non-parametric analysis were used. Data are given as mean ± s.e.m.

Results

A decrease in the disability score was observed up to the second week. The difference was significant compared with the values of the baseline period. After the second week no further change was observed (Table 1). However, although the total score changed significantly, the reductions of subscales for rigidity and walking, did not reach significance (Table 1).

The greatest effects were seen in the total score using UPDRS (Table 1). All of the subscales also changed significantly.

The opinions of participants distributed among the categories used were: worsened: 1, not changed: 3, moderate improvement: 8, marked improvement: 3.

Side-effects were observed in two cases; these were dry mouth, mild excitation and palpitations. The concentrations of theophylline in the serum of both cases were nearly 10 mg L^{-1}. After decreasing the dose of theophylline to 75 mg day^{-1}, the side-effects ceased. There was no worsening of the parkinsonian symptoms in these patients.

The concentration of theophylline in serum was 4·44 ± 0·91 mg L^{-1} after one week of treatment, but this declined with continued therapy (Table 1).

Discussion

In spite of the introduction of levodopa therapy in Parkinson's disease, the long-term effective treatment of this disease has not been solved. New therapeutic approaches tend to delay the introduction of levodopa by stimulating endogenous dopamine

Correspondence: T. W. Stone, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, UK.

Table 1. Change in the graded rating scale during theophylline therapy (150 mg day^{-1}).

		Weeks of treatment					
	Baseline	1	2	3	4	8	12
Graded Clinical Rating Scale							
Total score	31·07 ± 2·1	27·53 ± 1·53*	24·87 ± 1·59*	24·73 ± 1·89*	24·60 ± 1·93*	24·50 ± 2·0*	23·90 ± 1·95*
Rigidity	8·27 ± 0·98	8·2 ± 0·97	6 ± 0·97	6·27 ± 0·92	5·47 ± 1·1	—	6·01 ± 1·19
Walking	8·67 ± 1·02	7·07 ± 0·73	6·87 ± 0·75	6·93 ± 0·97	6·93 ± 0·75	—	7·0 ± 1·02
Unified Parkinson's Disease Rating Scale							
Total score	38·73 ± 3·38	31·73 ± 1·9*	27·47 ± 2·3**	25·80 ± 1·97**	27·4 ± 2·44**	27·93 ± 2·70**	26·80 ± 2·51**
Mental	1·87 ± 0·38	1·4 ± 0·32*	0·27 ± 0·12**	0·67 ± 0·16*	0·67 ± 0·19*	0·47 ± 0·17*	0·51 ± 0·12*
Daily activities	14·80 ± 2·55	11·13 ± 1·03	9·53 ± 1·06*	10·07 ± 1·28*	10·2 ± 1·35*	10·20 ± 1·57*	10·57 ± 1·97*
Motor	22·07 ± 1·75	19·2 ± 1·3	17·67 ± 1·36	17·27 ± 1·66*	17·2 ± 1·53*	18·13 ± 1·51	17·50 ± 1·05*
Serum theophylline concn (mg L^{-1})							
	—	4·44 ± 0·91	3·98 ± 0·76	2·81 ± 0·47	2·90 ± 0·51	—	—

Values are shown as mean ± s.e.m. Statistical significance from pre-theophylline (baseline) scores are indicated by $*P < 0·05$, $**P < 0·01$.

release or its receptors, or by interfering with amino acid-releasing neurons which probably affect indirectly the release of dopamine (Turski et al 1990; Magyar 1993; The Parkinson Study Group 1993). Inhibition of endogenous dopamine release can be induced by activation of presynaptic D_2 receptors, and by the stimulation of adenosine A_1 receptors (Michaelis et al 1979; Wood et al 1989; Cass & Zahniser 1991; Chowdhury & Fillenz 1991). Blockade of these should, therefore, enhance dopamine release.

Data presented in this study show that low doses of the non-selective adenosine receptor antagonist theophylline (150 mg day^{-1}), can improve parkinsonian symptoms. Both scales used revealed clear and significant improvement after two weeks treatment with theophylline. It appears that the effect of theophylline can develop when the serum theophylline concentrations are under 5 mg L^{-1}. Higher concentrations in two cases caused side-effects. A decrease in disability scores was evident in all UPDRS subscales as well as total scores, while significant changes were seen using the total score derived from our own criteria (Mally 1992). More than half of the patients involved in the present study reported subjective improvement, which developed after two weeks treatment with theophylline and lasted for the following three months. The persistence of improvement over the 3-month period makes it very unlikely that a placebo response was responsible.

It is interesting to note that the improvement in motor scores was accompanied by an improvement in mental well-being. It is impossible to say at present whether the improved motor performance was responsible for the elevation in mood or whether the converse applies.

Data from this study showed that a low dose of theophylline improved the symptoms of Parkinson's disease. This effect of theophylline may be based on the non-selective inhibition of adenosine receptors. Previous studies have demonstrated that A_{2a} receptors are localized to striatum, nucleus accumbens and olfactory tubercle (Jarvis & Williams 1988; James et al 1992). Even following degeneration of dopaminergic projections to striatum seen in Parkinson's disease, or after administration of 6-hydroxydopamine to animals (Martinez-Mir et al 1991), the number of A_{2a} receptors was not changed. It could be proposed that A_{2a} receptors are thus confined to postsynaptic elements; interactions between adenosine and dopamine mechanisms could take place at the level of enkephalinergic cells or cholinergic neurons where they could exert opposite effects (Vellucci et al 1993). Although the precise site and nature of the interaction remains unclear, it is now known that A_2 receptors can suppress postsynaptic dopamine-receptor activation, as revealed by the modification of binding of dopamine to postsynaptic D_2 receptors (Ferre et al 1991). The effect of theophylline in Parkinson's disease may thus be realized through these postsynaptic sites as well as via an enhancement of release.

In summary, this preliminary open study draws attention to the finding that theophylline, in low doses, induced a significant improvement in Parkinson's disease patients' symptoms within two weeks, and lasted throughout the treatment period of three months. Theophylline could, therefore, prove to be a safe, useful adjunct in the therapy of Parkinson's disease.

References

Barraco, R. A., Martens, K. A., Parizon, M., Normile, H. J. (1993) Adenosine A_{2a} receptors in the nucleus accumbens mediate locomotor depression. Brain Res. Bull. 34: 397–404

Cass, W. A., Zahniser, N. R. (1991) Potassium channel blockers inhibit D_2 dopamine, but not A_1 adenosine receptor mediated inhibition of striatal dopamine release. J. Neurochem. 57: 147–152

Chowdhury, M., Fillenz, M. (1991) Presynaptic adenosine A_2 and NMDA receptors regulate dopamine synthesis in rat striatal synaptosomes. J. Neurochem. 56: 1783–1788

Ferre, S., von Euler, G., Johansson, B., Fredholm, B. B., Fuxe, K. (1991) Stimulation of high-affinity adenosine A_2 receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes. Proc. Natl. Acad. Sci. USA 88: 7238–7241

Hoehn, M. M., Yahr, M. D. (1967) Parkinsonism: onset, progression and mortality. Neurology 17: 427–442

James, S., Xuereb, J. H., Askalan, R., Richardson, P. J. (1992) Adenosine receptors in post-mortem human brain. Br. J. Pharmacol. 105: 238–244

Jarvis, M. F., Williams, M. (1988) Differences in adenosine A_1 and A_2 receptors density revealed by autoradiography. Pharmacol. Biochem. Behav. 30: 707–714

Lang, A. E., Fahn, S. (1989) Assessment of Parkinson's disease. In: Munsat, T. L. (ed.) Quantification of Neurologic Deficity. Butterworth, Boston, pp 285–309

Mally, J. (1992) Some new aspects of the effect of (−)deprenyl in Parkinson's disease—a retrospective study. J. Neural Transm. 4: 155–164

Magyar, K. (1993) Pharmacology of monoamine oxidase type B inhibitors. In: Szelenyi, I. (ed.) Inhibitors of Monoamine Oxidase B: Pharmacology and Clinical Use in Neurodegenerative Disorders. Birkhauser Verlag, Basel, pp 125–143

Martinez-Mir, M. I., Probst, A., Palacios, J. M. (1991) Adenosine A_2 receptors: selective localization in the human basal ganglia and alterations with disease. Neuroscience 42: 697–706

Michaelis, M. L., Michaelis, E. K., Myers, S. L. (1979) Adenosine modulation of synaptosomal dopamine release. Life Sci. 24: 2083–2092

The Parkinson Study Group (1993) Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. N. Engl. J. Med. 328: 176–183

Turski, L., Klockgether, T., Turski, W. A., Schwarz, M., Sontag, K. (1990) Blockade on excitatory neurotransmission in the globus pallidus induces rigidity and akinesia in the rat: implications for excitatory neurotransmission in pathogenesis of Parkinson's disease. Brain Res. 512: 125–131

Vellucci, S. V., Sirinathsinghji, D. J. S., Richardson, P. J. (1993) Adenosine A_2 receptor regulation of apomorphine-induced turning in rats with unilateral striatal dopamine denervation. Psychopharmacology 111: 383–388

Wood, P. L., Kim, H. S., Boyar, W. C., Hutchinson, A. (1989) Inhibition of nigrostriatal release of dopamine in the rat by adenosine receptor agonists: A_1 receptor mediation. Neuropharmacology 28: 21–25

J. Pharm. Pharmacol. 1994, 46: 517–518
Received September 10, 1993
Accepted November 29, 1993

The effect of low temperature on the enzyme activities and the level of SH groups in benign gastric ulcer and gastric carcinoma

J. RZYMOWSKA, *Department of Cell Biology, M. Skłodowska-Curie University, Lublin, Poland*

Abstract—Homogenates of human benign gastric ulcers and gastric carcinomas were stored at a low temperature (4°C). The activities of L-lactate dehydrogenase (LDH), malic dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase (GR) and the concentrations of SH-containing compounds showed changes. Increased activities of LDH, MDH, G6PDH and GR occurred in the gastric carcinomas stored at 4°C compared with controls at 37°C. In contrast, the activities of G6PDH and GR decreased in the tissues of benign gastric ulcers. On incubation at 4°C the SH-containing compounds incorporated into protein molecules, SH groups binding with protein and non-protein SH groups decreased in both the benign and malignant tissues.

Very little is known about the response of the gastric mucosa or the susceptibility of the stomach to carcinogenesis at low temperatures. Malignant tissues represent unrestricted growth not controlled by the growth regulation mechanisms in the organs.

This study examined the effect of prolonged hypothermia, persisting from 1 to 72 h, on the activities of several enzymes and on the SH groups, in benign gastric ulcer and gastric carcinoma. I attempted to discover whether low temperature could be used as a therapeutic agent to control tumour cell growth.

Materials and methods

Postoperation material (benign gastric ulcer and gastric carcinoma) was used. Tissues were obtained from patients who underwent surgery for ulcers and advanced gastric carcinoma at the Surgical Clinic in Lublin. Fragments of the tissues were incubated in air, in Eagle's minimum essential medium (MEM 1959) at 4°C for 1, 5, 10, 24, 48 and 72 h. Control tissues were incubated at 37°C. For determination of the enzyme activities and levels of SH groups, the weighed tissue was cut into small pieces and transferred to cold homogenizing vessels containing homogenization buffer. The homogenates were then centrifuged, and the supernatant was filtered through glass wool to remove surface lipids.

The content of the protein was estimated in homogenate samples according to Bradford's method (Bradford 1976). Samples were also taken for determination of activities of the following enzymes: L-lactate dehydrogenase (LDH), malic dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH) and glutathione reductase (GR) (Krawczyński 1972; Szczeklik 1974). The levels of SH groups incorporated into protein molecules (P-SH), SH binding with protein (PB-SH) and non-protein SH (NP-SH) were determined.

The enzyme activities were presented as nkat (units × 16·67) (mg protein)$^{-1}$ and the level of SH groups as nmol (mg protein)$^{-1}$ (Ellman 1959; Truscott & Augusteyn 1977).

All results were analysed using Student's *t*-test.

Results and discussion

Tissue sections of pathological gastric mucosa obtained from stomachs surgically resected, were incubated at 4 and 37°C. The sections incubated at 37°C served as controls.

On the basis of these experiments it was observed that the activities of LDH, MDH, G6PDH and GR in cancer tissues increased at 4°C, and those of MDH, G6PDH and GR in benign gastric ulcer tissues decreased in comparison with control cultures (Table 1).

The level of SH groups, whether incorporated into protein molecules, bound to protein or free, increased in the carcinoma cells (Table 2).

Two tissues were thought to be the sites of lactate metabolism. The possibility that high levels of L-lactate may slowly be reduced by tumour cells should not be ignored. The production and subsequent reoxidation of L-lactate is dependent not only upon the concentration of the substrate and product but also on the reaction properties of the enzyme. LDH is an indicator of the presence of metastatic cancer. It is important to note that a high activity of LDH occurs in a number of cancer types and it may be a helpful parameter in cancer without any other markers (Petrelli et al 1985). Malignant changes are associated in all organs with a considerable increase of the slowest migrating LDH isozymes (Manly et al 1987). The MDH activity was found to decrease in the ulcer cells in relation to carcinoma cells. A significantly higher level of G6PDH activity was found in carcinoma compared with ulcer cells. The results suggest that the determination of G6PDH activity could be a valuable method to distinguish ulcers from tumours. It is possible that intensification of G6PDH activity in cancer is a sign of the shift of the carbohydrate metabolism from the aerobic pathway, or that the activity of pentose is higher in tumour cells because of an increased need for nucleic acid precursors in tissues with faster growth rates. The results which showed a higher level of G6PDH activity in carcinoma than in ulcer cells are in accordance with the reports of other authors (Weber 1977; Bokun et al 1987). It is likely that glutathione reductase acts to protect critical sulph-