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The timing of administration, dose dependence and efficacy of dopa decarboxylase inhibitors on the reversal of motor disability produced by L-DOPA in the MPTP-treated common marmoset

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ABSTRACT

Dopa decarboxylase inhibitors are routinely used to potentiate the effects of L-DOPA in the treatment of Parkinson's disease. However, neither in clinical use nor in experimental models of Parkinson's disease have the timing and dose of dopa decarboxylase inhibitors been thoroughly explored. We now report on the choice of dopa decarboxylase inhibitors, dose and the time of dosing relationships of carbidopa, benserazide and L-α-methyl dopa (L-AMD) in potentiating the effects of L-DOPA in the 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)-treated common marmoset. Pre-treatment with benserazide for up to 3 h did not alter the motor response to L-DOPA compared to simultaneous administration with L-DOPA. There was some evidence of a relationship between carbidopa and benserazide dose and increased locomotor activity and the reversal of motor disability. But in general, commonly used dose levels of dopa decarboxylase inhibitors appeared to produce a maximal motor response to L-DOPA. In contrast, dyskinesia intensity and duration continued to increase with both carbidopa and benserazide dose. The novel dopa decarboxylase inhibitor, L-AMD, increased locomotor activity and improved motor disability to the same extent as carbidopa or benserazide but importantly this was accompanied by significantly less dyskinesia. This study shows that currently, dopa decarboxylase inhibitors may be routinely employed in the MPTP-treated primate at doses which are higher than those necessary to produce a maximal potentiation of the anti-parkinsonian effect of L-DOPA. This may lead to excessive expression of dyskinesia in this model of Parkinson's disease and attention should be given to the dose regimens currently employed.

1. Introduction

The dopa decarboxylase inhibitors, carbidopa and benserazide, are routinely used in the treatment of Parkinson's disease to prevent peripheral metabolism of L-DOPA to dopamine (Da Prada et al., 1987). This allows a lowering of L-DOPA dose and increases the availability of L-DOPA to the brain. Administration of L-DOPA without dopa decarboxylase inhibitors would lead to peripheral dopamine formation but also to the formation of noradrenaline and adrenaline and consequently dopa decarboxylase inhibitors lead to a decrease in cardiovascular side-effects and notably hypotension.

However, because dopa decarboxylase inhibitors were first introduced into therapy several decades ago, the rationale for the timing of administration, duration of effect relative to L-DOPA, the dose of dopa decarboxylase inhibitors required and the relative potency of carbidopa and benserazide are not well defined (Hadjiconstantinou and Neff, 2008; Jonkers et al., 2001).

Carbidopa and benserazide are typically administered simultaneously with each dose of L-DOPA. This presumes that the onset of decarboxylase activity inhibition occurs sufficiently rapidly to prevent peripheral L-DOPA metabolism and that inhibition persists during the time course of effect of L-DOPA (Pinder et al., 1976). The plasma half-lives (t1/2) of carbidopa and benserazide are approximately 2.5 h and this correlates with the extent of decarboxylase activity inhibition (Da et al., 1987; Lieberman et al., 1975; Korten et al., 1975). However, carbidopa pre-treatment was shown to reduce the rate of intravenous L-DOPA infusion required to achieve therapeutic L-DOPA plasma levels compared to simultaneous carbidopa treatment (Nutt et al., 1985). Similarly, in normal rats, carbidopa administered 30 min prior to L-DOPA (i.v. and i.p.) produced a significant increase in the AUC (area under the curve) and half-life for L-DOPA in plasma compared to simultaneous administration (Leppert et al., 1988). This suggests that prior inhibition of DDC is more effective for carbidopa. However, there are no similar studies on the most efficient timing of benserazide administration.

Both carbidopa and benserazide are commonly used in the same fixed 1:4 ratio with L-DOPA in Parkinson's disease but the reasons for this are not clear. This is despite *in vitro* and *in vivo* evidence that,

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benserazide is a more potent decarboxylase inhibitor than carbidopa by a factor of up to 30 based on enzyme analysis (Da et al., 1987). Clinical studies did not show any overall significant difference in clinical benefit between the two dopa decarboxylase inhibitors (Marsden et al., 1973; Admani et al., 1985). However, higher plasma levels of L-DOPA were achieved when administered in conjunction with benserazide compared to carbidopa, although the doses of dopa decarboxylase inhibitors used may not have been equivalent (Hagan et al., 1980).

In experimental models of Parkinson's disease there is little evidence of how dopa decarboxylase inhibitors should be used in conjunction with L-DOPA. There have not been any recent investigations of the timing and dosage of dopa decarboxylase inhibitors in either 6-OHDA lesioned rats or 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)-treated primates. In fact, the doses and timings for dopa decarboxylase inhibitor use employed in the MPTP-treated primate vary markedly between laboratories and largely appear historical in nature (Iravani et al., 2006; Campos-Romo et al., 2009; Samadi et al., 2008; Quik et al., 2002; Close et al., 1985; Fox et al., 2002). In this model, carbidopa and benserazide doses range between 1 and 50 mg with both concomitant and pre-treatment protocols being utilized. As a consequence, it is unknown whether these dosing regimens result in an optimal potentiation of the effects of L-DOPA or not.

As a consequence, we have studied the effects of a range of doses of carbidopa and benserazide currently employed in primate studies on the actions of L-DOPA in the MPTP-treated common marmoset. In addition, we have examined the effects of L-AMD (L-alpha methyl dopa) as a potential dopa decarboxylase inhibitors for use in Parkinson's disease (Yitey-Smith and Varma, 1970; Hess et al., 1961).

2. Methods and materials

2.1. Common marmosets

Male and female adult common marmosets, $n=6$, (350 g or above; Harlan UK) were housed alone or in pairs on a 12 h light/dark cycle, 50% humidity at a temperature of 25 ± 1 °C. Animals had *ad libitum* access to Mazuri food pellets and water. In addition, they received mashed up Mazuri pellets, forage mix and pumpkin seeds in the morning and fresh fruits in the afternoon. All procedures were carried out in accordance with Home Office regulations under the Animals (Scientific Procedures) Act 1986 and project licence 70/6345. The investigation was approved by King's College London Ethical Committee.

2.2. MPTP administration

Motor deficits were induced by the administration of MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine HCl 2.0 mg/kg, s.c.; Sigma Chemical, UK) in sterile 0.9% saline (Baxter Healthcare Ltd.), daily for up to 5 consecutive days. The MPTP-treatment regime employed for these animals produces 85–90% degeneration of nigral dopaminergic neurons, a "full lesion" and representative of late stage Parkinson's disease in man (Jenner and Marsden, 1986).

The animals developed marked motor deficits consisting of reduced locomotor activity, a slowness or lack of movement and postural and co-ordination deficits. They were hand fed until they regained the ability to feed and groom independently. At the time of the experiments the animals had recovered from the acute effects of MPTP treatment and exhibited stable motor deficits and reduced locomotor activity as previously reported (Pearce et al., 1995).

2.3. Induction of dyskinesia

MPTP-treated common marmosets were 'primed' to exhibit dyskinesia by the twice daily administration of L-DOPA (12.5 mg/kg, p.o.) plus carbidopa (12.5 mg/kg, p.o.) for up to 28 days as previously reported (Pearce et al., 1995; Jackson et al., 2007). The animals progressively developed chorea, dystonia and athetosis which became stable and was reproducible with subsequent L-DOPA treatment (Pearce et al., 1995). Prior to this investigation, the animals had previously been treated with L-DOPA, dopamine agonists and dopa decarboxylase inhibitors but had not received any drug treatment for a period of at least one month.

2.4. Investigation of the effect of timing of dopa decarboxylase inhibitor administration

The duration of benserazide induced DDC inhibition was measured in MPTP-treated common marmosets by administering benserazide (10 mg/kg) at 60, 120 or 180 min prior to L-DOPA (12.5 mg/kg) or L-DOPA and benserazide administered concomitantly. Animals received at least a 6 day washout period between treatments.

2.5. Investigation of the effect of dose of dopa decarboxylase inhibitors on motor function

To assess the effect of different doses of dopa decarboxylase inhibitors on L-DOPA-induced locomotor activity, reversal of motor disability and dyskinesia, the effects of L-DOPA alone or administered concomitantly with either carbidopa (3.125, 6.25, 9.375 or 12.5 mg/kg) or benserazide (1.0, 3.125, 6.25, 9.375 or 12.5 mg/kg) were studied. Animals were placed into test cages to acclimatize for 1 h and were then treated simultaneously with L-DOPA and either carbidopa or benserazide or L-DOPA alone.

2.6. Investigation of the effects of L-AMD on L-dopa induced motor function

Animals were placed into test units. Following a 1 h acclimatisation period, animals were treated with L-DOPA (12.5 mg/kg) or L-DOPA in combination with carbidopa, benserazide or L-AMD (12.5 mg/kg). L-AMD has a similar chemical structure to both carbidopa and benserazide and this together with the availability of established, historical data for carbidopa (12.5 mg/kg) in this model, assisted in the determination of L-AMD dosage to be used.

2.7. Behavioural assessments

All assessments were made in a blinded manner by trained observers through a one way mirror with animals housed in a sound-reduced room. After acclimatization and drug treatment the animals were monitored for 5 h. For the study where the effect of pre-treatment with a dopa decarboxylase inhibitor was investigated, details of the timings of drug administration are given in the figure legends.

2.8. Locomotor activity

Basal assessment of locomotor activity took place during the acclimatisation period. Locomotor activity was assessed using automated test units fitted with 8 photoelectric switches whereby interruption of a photo-electric switch (infra red beam measuring activity at 8 Hz) was automatically recorded as a single locomotor count. Locomotor counts were accumulated and plotted as total counts per 30 min over the course of the test day to produce a time course of drug activity. From the time course data, the area under the curve (AUC) was calculated and represents total locomotor activity over the duration of the experiment.

2.9. Motor disability

Basal assessment took place in the activity units during the acclimatisation period. Subsequently, assessment of motor disability