Levetiracetam Potentiates the Antidyskinetic Action of Amantadine in the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-Lesioned Primate Model of Parkinson’s Disease

Michael P. Hill, Paula Ravenscroft, Erwan Bezard, Alan R. Crossman, Jonathan M. Brotnie, Anne Michel, Renee Grimée, and Henrik Klitgaard

Motac Neuroscience Ltd, Manchester, United Kingdom (M.P.H., P.R., A.R.C.); Basal Gang, Laboratoire de Neurophysiologie, Université Victor Segalen-Bordeaux 2, Bordeaux, France (E.B.); University of Manchester, School of Biological Sciences, Manchester, United Kingdom (A.R.C.); Toronto Western Research Institute, Toronto Western Hospital, Toronto, Canada (J.M.B.); and UCB S.A., Pharma Sector, Preclinical CNS Research, Braine L’Alleud, Belgium (A.M., R.G., H.K.)

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ABSTRACT

Levetiracetam (LEV) (Keppra; UCB Pharma, Brussels, Belgium) has recently been reported to have antidyskinetic activity against levodopa (L-DOPA)-induced dyskinesia in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset and macaque models of Parkinson’s disease. Amantadine is frequently used as adjunctive therapy for L-DOPA-induced dyskinesia, but adverse effects limit its clinical utility. The current study was designed to investigate whether LEV can potentiate the antidyskinetic action of amantadine. The antiparkinsonian and antidyskinetic effects of LEV (13 and 60 mg/kg) and amantadine (0.01, 0.03, 0.1, and 0.3 mg/kg), administered alone and in combination, were assessed in the MPTP-lesioned marmoset model of L-DOPA-induced dyskinesia (n = 12). LEV (60 mg/kg) and amantadine (0.3 mg/kg) administered alone significantly reduced L-DOPA-induced dyskinesia without compromising the antiparkinsonian action of L-DOPA. Lower doses were without any significant effects. The combination of LEV (60 mg/kg) and amantadine (0.01, 0.03, 0.1, and 0.3 mg/kg) significantly decreased dyskinesia severity, without compromising the antiparkinsonian action of L-DOPA, more efficaciously than LEV or amantadine monotherapy. These results support the concept that normalization of different pathophysiological mechanisms (i.e., altered synchronization between neurons and enhanced N-methyl-D-aspartate transmission) has a greater efficacy. Combined LEV/amantadine therapy might be useful as an adjunct to L-DOPA to treat dyskinetic side effects and to expand the population of Parkinson’s disease patients who benefit from treatment with amantadine alone.

Abnormal involuntary movements, dyskinesia, represent a debilitating complication of levodopa (L-DOPA) therapy for Parkinson’s disease that is ultimately experienced by the vast majority of the patients (Cotzias et al., 1969; Stocchi et al., 1997). The neural mechanisms underlying L-DOPA-induced dyskinesia (LID) in Parkinson’s disease are far from clear, despite major advances made in recent years. The generation of dyskinesia has been associated with a sequence of events that include pulsatile stimulation of dopamine receptors, downstream changes in proteins and genes, and abnormalities in nondopaminergic transmitter systems (Bezard et al., 2001). These events combine to produce alterations in the neuronal firing patterns that signal between the basal ganglia and the cerebral cortex (Bezard et al., 2001).

Among the sequence of events, disordered striatal glutamatergic transmission plays a major role in both the genesis of parkinsonian symptoms and LID (Chase and Oh, 2000). Blockade of N-methyl-D-aspartate (NMDA) receptors potentiates L-DOPA action in monoamine-depleted rats (Klockgether and Turski, 1990), 6-hydroxydopamine-lesioned rats (Morelli et al., 1992), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned nonhuman primates (Wullner et al., 1992). NMDA receptor antagonism improves L-DOPA-induced motor complications in 6-hydroxydopamine-lesioned rats (Papa et al., 1995) and MPTP-lesioned nonhuman primates (Papa and Chase, 1996). The noncompetitive NMDA receptor antagonist amantadine exerts a mild antiparkinsonian effect (Klockgether and Turski, 1990). Thus, these observations support the concept that normalization of different pathophysiological mechanisms (i.e., altered synchronization between neurons and enhanced N-methyl-D-aspartate transmission) has a greater efficacy. Combined LEV/amantadine therapy might be useful as an adjunct to L-DOPA to treat dyskinetic side effects and to expand the population of Parkinson’s disease patients who benefit from treatment with amantadine alone.
nian action in patients (Butzer et al., 1975) and significantly reduces LID in MPTP-lesioned nonhuman primates (Blanchet et al., 1998). Such an antidyskinetic effect has been confirmed in Parkinson’s disease patients with LID (VerHagen Metman et al., 1998a; VerHagen Metman et al., 1998b; Snow et al., 2000; Del Dotto et al., 2001). Unfortunately, the induction of unwanted adverse effects detract markedly from the clinical utility of amantadine (Hayden et al., 1981; Macchio et al., 1993). This highlights the need for identifying alternative strategies that could permit the dose of amantadine to be reduced.

Dyskinesias also have been hypothesized to reflect a pathological synchronization/desynchronization process (Boraud et al., 2002) based on the relationship between altered firing patterns and changes in the level of synchronization of neurons of the internal segment of the globus pallidus, and the occurrence of dyskinesia (Boraud et al., 2001; Heimer et al., 2002). Indeed, it is antagonism of this pattern of activity that has been proposed as the mechanism of action of pallidotomy or pallidal deep brain stimulation in reducing LID. We have previously shown that levetiracetam (LEV) (Keppra; UCB Pharma, Brussels, Belgium) has antidyskinetic activity similar to pallidal lesion/stimulation in MPTP-lesioned primate models of Parkinson’s disease (Hill et al., 2003; Bezarad et al., 2004). LEV is a novel antiepileptic drug with proven efficacy as adjunctive therapy in adult patients with refractory partial epilepsy (Marson et al., 2001) and a unique ability to reduce neuronal (hyper)synchronization in animal models of epilepsy (Margineanu and Klitgaard, 2000; Margineanu and Kiltgaard, 2002; Kiltgaard et al., 2003).

We hypothesized that normalization of these two pathophysiological mechanisms would improve LID more than controlling one of them. Thus, the present study tested the hypothesis that the combination of amantadine and LEV, two drugs with very different profiles and modes of action, would provide synergistic or additive antidyskinetic activity in the MPTP-lesioned marmoset model of Parkinson’s disease.

Materials and Methods

Animals. Experiments were performed on 12 nondrug naive adult common marmosets (Callithrix jacchus; 300-350 g; 6 male, 6 female) from a closed colony in the United Kingdom in accordance with the requirements of The Animals (Scientific Procedures) Act, 1986. The animals were kept in controlled housing conditions, with constant temperature (25°C), relative humidity (50%), and 12-h light/dark cycles (8:00 AM lights on). The animals had free access to food (Masuri primate diet-E; Scientific Dietary Services, Manchester, UK), fresh fruit supplements, and water. Marmosets were rendered parkinsonian by administration of MPTP-hydrochloride (Sigma-Aldrich, St. Louis, MO; 2 mg/kg s.c. for 5 consecutive days) as previously described (Hill et al., 2003; Savola et al., 2003). Following stabilization of the parkinsonian state, the animals were treated with Madopar dispersible (Roche Applied Science, Neuilly, France) (equivalent to 12 mg/kg l-DOPA and 3 mg/kg benserazide) twice daily for 6 weeks as previously described (Hill et al., 2003; Savola et al., 2003), during which time they developed LID. Time between MPTP intoxication and present experiments ranges from 18 (n = 6) to 24 months (n = 6).

Study Design. The dose of l-DOPA was tailored for each individual animal (i.e., 15 or 17.5 mg/kg) in order to elicit an optimal level of reversal of parkinsonian symptoms in all animals. All drugs were administered orally in the animals’ home cage in the following combinations: l-DOPA/vehicle, l-DOPA/LEV (13 mg/kg and 60 mg/kg), l-DOPA/amantadine (0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, and 0.3 mg/kg), and l-DOPA/LEV (13 mg/kg and 60 mg/kg/amantadine 0.03 mg/kg, 0.1 mg/kg, and 0.3 mg/kg). Both LEV and amantadine were provided by UCB S.A. The animals were then placed in observation cages. They were not disturbed during the observation period, and their behavior was videotaped for 6 h. A minimum of 48 h was allowed between drug administrations to individual animals.

Behavioral Assessments. A battery of behavioral tests was performed as previously described (Hill et al., 2003; Savola et al., 2003). A quantitative assessment of locomotor activity using computer-based passive infrared activity monitors (Excalibur, modified by the Central Electronic Workshop University of Manchester, UK) was obtained every 5 min for the duration of the experiment. Nonparametric measures of parkinsonism based on range of movement (0–9), bradykinesia (0–3), and posture (0–1) scales were made, by post hoc analysis of video-recordings by an observer blinded to the treatment, in 10-min observation periods every 30 min throughout the duration of the experiment. The parkinsonian disability score was a combination of the range of movement, bradykinesia, and posture scores according to the formula (18 + ((bradykinesia × 3) + (posture × 9)) – (range of movement × 2)) to give a global parkinsonian disability rating (Hill et al., 2003; Savola et al., 2003). Nonparametric measures of dyskinesia severity based on the following scale were made, by post hoc analysis of video-recordings, in 10-min observation periods every 30 min throughout the duration of the experiment (Hill et al., 2003; Savola et al., 2003). Dyskinesias, broadly categorized into chorea (hyperkinetic, purposeless dance-like movements) and dystonia (sustained, abnormal muscle contractions), were rated as 0 = absent; 1 = mild, fleeting, present less than 30% of the observation period; 2 = moderate, not interfering with normal activity, present more than 30% of the observation period; 3 = marked, at times interfering with normal activity, present less than 70% of the observation period; and 4 = severe, present more than 70% of the observation period, essentially replacing normal activity.

Statistical Analysis. Data for parkinsonian disability and dyskinesia were cumulated for each 1-h period and analyzed with a nonparametric repeated measures one-way analysis of variance (ANOVA) (Friedman test; Fr) followed by Dunn’s multiple comparison test (Graphpad Prism version 3). Cumulated activity data were plotted as mean ± S.E.M. and statistical analysis was performed using a parametric repeated measures one-way ANOVA followed by Tukey’s multiple comparison’s test (Graphpad Prism version 3). As the study design was complex in terms of the number of treatment combinations employed and time bins analyzed, we have reported only the significant and most relevant effects seen to facilitate the reading of the manuscript.

Results

In the time periods 0 to 1 h, 1 to 2 h, and 2 to 3 h, whatever the treatment considered, there was a significant effect of treatment on dyskinesia (Fr = 56 with 14 df, Fr < 0.01; Fr = 75.38 with 14 df, Fr < 0.001; Fr = 38 with 14 df, Fr < 0.001, respectively). In the time periods 0 to 1 h and 2 to 3 h, whatever the treatment considered, there was a significant effect of treatment on parkinsonian disability (Fr = 38.97 with 14 df, Fr < 0.001; Fr = 46.46 with 14 df, Fr < 0.001, respectively). There was no effect of treatment on parkinsonian disability during the 1- to 2-h time period (Fr = 22.87 with 14 df, Fr > 0.05). Since dyskinesia was present for the first 3 h only, no data are presented for time periods 3 to 4, 4 to 5, and 5 to 6 h.

Effect of l-DOPA. l-DOPA alone alleviated parkinsonian symptoms, which was accompanied by dyskinesia (Fig. 1,
A–F) that was characterized by a mixture of chorea and dystonia. Median severity of dyskinesia was of a “mild” level during the 0- to 1-h time period (Fig. 1A), a “severe” level during the 1- to 2-h time period (Fig. 1C), and a “moderate” level during the 2- to 3-h time period (Fig. 1E). Dyskinesia was absent during subsequent time periods (data not shown).

**Effect of LEV in Combination with L-DOPA.** Dyskinesia was significantly reduced following LEV (60 mg/kg)/L-DOPA combination therapy, but not following LEV (13 mg/kg)/L-DOPA combination, during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, compared with L-DOPA alone (*P < 0.05, **P < 0.01, and ***P < 0.001, respectively; Fig. 1, A, C, and E).
antiparkinsonian action of L-DOPA was not impaired by either dose of LEV (Fig. 1, B, D, and F). Total activity counts (i.e., 0–6 h), derived from locomotor activity measurement (Fig. 1G), following LEV (13 mg/kg)/L-DOPA and LEV (60 mg/kg)/L-DOPA combination therapy were significantly reduced by 41% and 42%, respectively, compared with L-DOPA alone (P < 0.001 for both; Fig. 1H).

**Effect of Amantadine in Combination with L-DOPA.** Amantadine (0.01–0.3 mg/kg)/L-DOPA combination therapy had no significant effect on LID or parkinsonian disability at any time point post drug administration (Fig. 2, A–F). Following amantadine (0.3 mg/kg)/L-DOPA combination therapy, dyskinesia was significantly reduced during the 1- to 2-h time period, compared with L-DOPA alone (P < 0.01; Fig. 2C), without affecting the antiparkinsonian action of L-DOPA (Fig. 2D). Total activity counts (i.e., 0–6 h) following amantadine (0.03 mg/kg)/L-DOPA, amantadine (0.1 mg/kg)/L-DOPA, and amantadine (0.3 mg/kg)/L-DOPA combination therapy were significantly reduced by 53, 45, and 62%, respectively, compared with L-DOPA alone (all P < 0.001; Fig. 2H).

**Effect of LEV (13 mg/kg) and Amantadine (0.01–0.3 mg/kg) in Combination with L-DOPA.** LEV (13 mg/kg)/amantadine (0.01 mg/kg)/L-DOPA combination and LEV (13 mg/kg)/amantadine (0.03 mg/kg)/L-DOPA combination therapies had no significant effect on dyskinesia or parkinsonian disability at any time period post drug administration, compared with L-DOPA alone (Fig. 3, A–F).

Dyskinesia was significantly reduced following LEV (13 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy during the 1- to 2-h time period, compared with L-DOPA alone (P < 0.01, Fig. 3C). The percentage of dyskinesia reduction was 47% following LEV (13 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy, whereas it was only reduced by 20% following LEV (13 mg/kg)/L-DOPA and 7% following amantadine (0.1 mg/kg)/L-DOPA during the 1- to 2-h time period. The antiparkinsonian action of L-DOPA was preserved at any time period post drug administration (Fig. 3, B, D, and F). Total activity counts (i.e., 0–6 h) following LEV (13 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy were significantly reduced by 42%, compared with L-DOPA alone (P < 0.001; Fig. 3H), but not significantly different from L-DOPA/amantadine (0.1 mg/kg) combination therapy or L-DOPA/LEV (13 mg/kg) combination therapy (Fig. 3H).

Dyskinesia was significantly reduced following LEV (13 mg/kg)/amantadine (0.3 mg/kg)/L-DOPA combination therapy during the 1- to 2-h time period, compared with L-DOPA alone (P < 0.01; Fig. 3C). The antiparkinsonian action of L-DOPA was preserved at any time period post drug administration (Fig. 3, B, D, and F). Total activity counts (i.e., 0–6 h) following LEV (13 mg/kg)/amantadine (0.3 mg/kg)/L-DOPA combination therapy were significantly reduced by 50%, compared with L-DOPA alone (P < 0.001; Fig. 3G), but not significantly different from L-DOPA/amantadine (0.3 mg/kg) combination therapy or L-DOPA/LEV (13 mg/kg) combination therapy (Fig. 3G).

**Effect of LEV (60 mg/kg) and Amantadine (0.01–0.3 mg/kg) in Combination with L-DOPA.** Dyskinesia was significantly reduced following LEV (60 mg/kg)/amantadine (0.01 mg/kg)/L-DOPA combination therapy during the 1- to 2- and 2- to 3-h time periods, compared with L-DOPA alone (both P < 0.05; Fig. 4, C and E). The percentages of dyskinesia reduction were 50%, 53%, and 89% following LEV (60 mg/kg)/amantadine (0.01 mg/kg)/L-DOPA combination therapy during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. In contrast, dyskinesia was reduced by 50%, 40%, and 71% following LEV (60 mg/kg)/L-DOPA and by 50%, 0%, and 56% following amantadine (0.01 mg/kg)/L-DOPA during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. The antiparkinsonian action of L-DOPA was preserved at any time period post drug administration (Fig. 4, B, D, and F). Total activity counts (i.e., 0–6 h) following LEV (60 mg/kg)/amantadine (0.01 mg/kg)/L-DOPA combination therapy were significantly reduced by 62% compared with L-DOPA alone (P < 0.001; Fig. 4H).

Dyskinesia was significantly reduced following LEV (60 mg/kg)/amantadine (0.03 mg/kg)/L-DOPA combination therapy during the 1- to 2- and 2- to 3-h time periods, compared with L-DOPA alone (both P < 0.01; Fig. 4, C and E). The percentages of dyskinesia reduction were 50, 53, and 100% following LEV (60 mg/kg)/amantadine (0.03 mg/kg)/L-DOPA combination therapy during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. In contrast, dyskinesia was reduced by 50%, 40%, and 71% following LEV (60 mg/kg)/L-DOPA and by 25, 7, and 22% following amantadine (0.03 mg/kg)/L-DOPA during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. The antiparkinsonian action of L-DOPA was preserved at any time period post drug administration (Fig. 4, B, D, and F). Total activity counts (i.e., 0–6 h) following LEV (60 mg/kg)/amantadine (0.03 mg/kg)/L-DOPA combination therapy were significantly reduced by 55%, compared with L-DOPA alone (P < 0.001; Fig. 4H), but not significantly different from L-DOPA/amantadine (0.03 mg/kg) combination therapy or L-DOPA/LEV (60 mg/kg) combination therapy (Fig. 4H).

Dyskinesia was significantly reduced following LEV (60 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, compared with L-DOPA alone (all P < 0.05; Fig. 4, A, C, and E). The percentages of dyskinesia reduction were 50, 73, and 78% following LEV (60 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. In contrast, dyskinesia was reduced by 50%, 40%, and 71% following LEV (60 mg/kg)/L-DOPA and 0, 7, and 33% following amantadine (0.1 mg/kg)/L-DOPA during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. The antiparkinsonian action of L-DOPA was preserved at any time period post drug administration (Fig. 4, B, D, and F). Total activity counts (i.e., 0–6 h) following LEV (60 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy were significantly reduced by 63%, compared with L-DOPA alone (P < 0.001; Fig. 4H) but not significantly different from L-DOPA/amantadine (0.1 mg/kg) combination therapy or L-DOPA/LEV (60 mg/kg) combination therapy (Fig. 4H).

Dyskinesia was significantly reduced following LEV (60 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, compared with L-DOPA alone (all P < 0.05; Fig. 4, A, C, and E). The percentages of dyskinesia reduction were 50, 73, and 78% following LEV (60 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. In contrast, dyskinesia was reduced by 50%, 40%, and 71% following LEV (60 mg/kg)/L-DOPA and 0, 7, and 33% following amantadine (0.1 mg/kg)/L-DOPA during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods, respectively. The antiparkinsonian action of L-DOPA was preserved at any time period post drug administration (Fig. 4, B, D, and F). Total activity counts (i.e., 0–6 h) following LEV (60 mg/kg)/amantadine (0.1 mg/kg)/L-DOPA combination therapy were significantly reduced by 63%, compared with L-DOPA alone (P < 0.001; Fig. 4H) but not significantly different from L-DOPA/amantadine (0.1 mg/kg) combination therapy or L-DOPA/LEV (60 mg/kg) combination therapy (Fig. 4H).
time periods, respectively. In contrast, dyskinesia was reduced by 50, 40, and 71% following LEV (60 mg/kg)/L-DOPA and by 25, 60, and 56% following amantadine (0.3 mg/kg)/L-DOPA during the 0- to 1-, 1- to 2-, and 2- to 3-h time periods. The antiparkinsonian action of L-DOPA was preserved at any time period post drug administration (Fig. 4, B, D, and F). Total activity counts (i.e., 0–6 h) following LEV (60 mg/kg)/amantadine (0.3 mg/kg)/L-DOPA combination therapy were significantly reduced by 50%, compared with L-DOPA alone (P < 0.001; Fig. 4H), but not
significantly different to L-DOPA/amantadine (0.3 mg/kg) combination therapy or L-DOPA/LEV (60 mg/kg) combination therapy (Fig. 4H).

Discussion

The main finding of this study was that the antidyskinetic effect of LEV and amantadine, two drugs with different mechanisms of action, is increased when given in combination. Notably, doses of LEV or amantadine that were not able to reduce LID when given alone with L-DOPA significantly reduced LID when administered in combination, without compromising the antiparkinsonian action of L-DOPA. These results suggest that combined LEV/amantadine therapy might be useful to treat LID in a larger population of Parkinson’s disease patients than is currently possible with amantadine alone.

Methodological Considerations. The total activity counts accumulated over the 6-h experimental period induced by L-DOPA alone reflect a combination of the antiparkinsonian action, hyperkinesias, and dyskinetic move-

Fig. 3. The effect of LEV (13 mg/kg) and amantadine (0.01–0.3 mg/kg) in combination with L-DOPA on dyskinesia and parkinsonian disability at 0 to 1 h (A and B), 1 to 2 h (C and D), 2 to 3 h (E and F), and on activity counts (G, time course; H, total activity) post drug administration in the MPTP-lesioned marmoset model of Parkinson's disease. Individual animal data and the median scores are shown on the graphs. **, P < 0.01 cf. L-DOPA + vehicle; ##, P < 0.01 cf. L-DOPA + amantadine (at the same dose, see Fig. 2), Friedman's test followed by Dunn's multiple comparisons test. Data are expressed as mean ± S.E.M. of 12 animals (G). ***, P < 0.001 cf. L-DOPA + vehicle; #, P < 0.01 cf. L-DOPA + amantadine (at the same dose, see Fig. 2), one-way repeated measures ANOVA followed by Tukey's test.
ments (Bezard et al., 2003). Thus, a reduction in the activity counts may account for the mix of these three parameters (Bezard et al., 2003; Iravani et al., 2003). The reduction of total activity counts observed following LEV (13 and 60 mg/kg) and amantadine (0.03, 0.1, and 0.3 mg/kg) administration, both alone and in combination, is most likely indicative of an antidyskinetic effect rather than a proparkinsonian effect since the parkinsonian disability score remained un-

Fig. 4. The effect of LEV (60 mg/kg) and amantadine (0.01–0.3 mg/kg) in combination with L-DOPA on dyskinesia and parkinsonian disability at 0 to 1 h (A and B), 1 to 2 h (C and D), 2 to 3 h (E and F), and on activity counts (G, time course; H, total activity) post drug administration in the MPTP-lesioned marmoset model of Parkinson’s disease. Individual animal data and the median scores are shown on the graphs. *, P < 0.05; **, P < 0.01; ***, P < 0.001 cf. L-DOPA + vehicle; #, P < 0.05; ##, P < 0.01 cf. L-DOPA + amantadine (at the same dose, see Fig. 2), Friedman’s test followed by Dunn’s multiple comparisons test. Data are expressed as mean ± S.E.M. of 12 (G). ***, P < 0.001 cf. L-DOPA + vehicle; ###, P < 0.001 cf. L-DOPA + amantadine (at the same dose, see Fig. 2), one-way repeated measures ANOVA followed by Tukey’s test.
changed, whereas the dyskinesia was reduced. The lack of proparkinsonian activity of LEV has been further confirmed in combination with a lower dose of L-DOPA (4 mg/kg) that produced a transient full reversal of parkinsonian motor abnormalities when administered on its own (data not shown).

The observed antidyskinetic effect is suggested to be relevant from a clinical perspective. The dyskinesia scale presently used focuses on the disability caused by dyskinesia rather than the amount of dyskinesia (Pearce et al., 1995; Brotchie and Fox, 1999). Thus, an improvement from a “severe” to “mild” level corresponds to a dyskinetic activity that was present more than 70% of the observation period, essentially replacing normal activity, to mild, fleeting dyskinesia, which was present for at least 30% of the observation period. If such actions could be transferred to the clinical setting, it would have a dramatic impact on patient’s quality of life.

Effect of Amantadine. In agreement with previous studies in nonhuman primates and Parkinson’s disease patients, amantadine reduced LID without affecting the antiparkinsonian action of L-DOPA (Verhagen Metman et al., 1998a; Verhagen Metman et al., 1998b; Snow et al., 2000; Del Dotto et al., 2001). Amantadine has been used for over 30 years as an antiparkinsonian agent. The highest dose used in the current study (0.3 mg/kg) is lower than that used in the clinic to treat Parkinson’s disease symptoms (100–400 mg/day) (Del Dotto et al., 2001). It is possible that the antidyskinetic action of amantadine is seen at lower doses than the antiparkinsonian action when considering a number of pharmacological actions of amantadine, including facilitation of presynaptic dopamine release (Mizoguchi et al., 1994; Takahashi et al., 1996), blockade of dopamine re-uptake (Mizoguchi et al., 1994), anticholinergic effects (Magazanik et al., 1996), and NMDA receptor antagonism (Kornhuber et al., 1991). Previous studies have suggested that the antiparkinsonian action of amantadine is most likely due to a combination of these effects, whereas the antidyskinetic action is most likely due to its NMDA receptor antagonist effect within the striatum, which is already reached at lower concentrations (Papa et al., 1995; Papa and Chase, 1996; Blanchet et al., 1998).

Effect of LEV. We have previously demonstrated that LEV has potential as an antidyskinetic agent against dyskinesia induced by L-DOPA or L-DOPA in combination with dopamine agonists in the MPTP-lesioned marmoset and macaque models of Parkinson’s disease (Hill et al., 2003; Bezard et al., 2004). In the current study, LEV (60 mg/kg) reduced LID in the 1st, 2nd, and 3rd hours post drug administration. This is in contrast to our previous studies where LEV was active only during the 1st hour post drug administration (Hill et al., 2003; Bezard et al., 2004). In addition, LEV revealed in this study an ability to reduce dyskinesia that was not at a “severe” level during the 1st and 3rd hours post drug administration. Again, this is in contrast to our previous studies where LEV was mainly active against “severe” dyskinesia.

The question of the mechanism of action still remains. We speculate that at least a part of the antidyskinetic activity of LEV may relate to desynchronisation of abnormal neuronal firing patterns since 1) abnormal synchronization of basal ganglia structures (Nini et al., 1995; Raz et al., 2000; Raz et al., 2001), a characteristic feature of the parkinsonian syndrome, is not normalized by dopamine replacement therapy (Heimer et al., 2002) and 2) LEV, contrary to other anti-epileptic drugs, is able to inhibit neuronal (hyper)synchronisation in animal models of epilepsy (Margineanu and Klitgaard, 2000; Margineanu and Klitgaard, 2002; Klitgaard et al., 2003). This suggests that LEV could potentiate the antidyskinetic effects of other therapies that might act upstream of the basal ganglia output nuclei. Amantadine is the only drug available for the treatment of LID. Although amantadine has been shown to be effective in the treatment of dyskinesia, many patients cannot use it due to tolerability problems (primarily central nervous system side effects including hallucinations, confusion, and nightmares) (Macchio et al., 1993). The proposed site of the antidyskinetic actions of amantadine is the striatum. This is upstream of the hypothesized site of action of LEV and suggests a theoretical mechanistic potential for additive effects against LID.

Additive Effect of LEV/Amantadine Combination. Combination of LEV with amantadine in the current study revealed an increased antidyskinetic action for several dose combinations in comparison with stand-alone administrations. An impressive example is the combination of LEV (13 mg/kg) and amantadine (0.1 mg/kg). This combination elicited a significant reduction in LID, with no significant reduction in the antiparkinsonian effect of L-DOPA, during the 2nd hour post drug administration, whereas LEV (13 mg/kg) or amantadine (0.1 mg/kg) on their own had no significant effect. However, more interesting is the fact that the combination of LEV (60 mg/kg) and amantadine (0.01, 0.03, 0.1, and 0.3 mg/kg) significantly decreased dyskinesia severity, without compromising the antiparkinsonian action of L-DOPA, more efficaciously than LEV or amantadine monotherapy. In terms of efficacy of antidyskinetic effect, the combinations of LEV (60 mg/kg) and amantadine (0.1 or 0.3 mg/kg) were most effective. Both these combinations induced a reduction in dyskinesia during the 1st, 2nd, and 3rd hours post drug administration. The magnitude of this effect is highlighted by the one effective dose of amantadine (0.3 mg/kg) reducing dyskinesia only during the second hour, post drug administration.

Conclusion

The current study demonstrates that the dose of amantadine necessary to reduce LID in MPTP-lesioned marmosets can be significantly reduced if administered in combination with LEV. The outcome is not only a more significant reduction in dyskinesia but also a prolonged duration of antidyskinetic action of amantadine. The most striking result was that combination of inactive doses of LEV and amantadine significantly reduced LID, without compromising the antiparkinsonian action of L-DOPA. This suggests that combined LEV/amantadine therapy may be superior to amantadine alone as an adjunct to L-DOPA to treat dyskinetic side effects in Parkinson’s disease patients.

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References


Address correspondence to: Henrik Klitgaard, UCB S.A. Pharma Sector, Preclinical CNS Research, Chemin du Foriest, B-1420 Braine-l’Alleud, Belgium. E-mail: henrik.klitgaard@ucb-group.com.