European Neuropsychopharmacology (2007) 17, 94–101

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# Isobolographic analysis of interactions between losigamone and conventional antiepileptic drugs in the mouse maximal electroshock model

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Received 23 November 2005; received in revised form 19 January 2006; accepted 14 February 2006

**KEYWORDS**
Losigamone;
Antiepileptic drugs;
Electroshock maximal;
Drug interactions;
Pharmacokinetic interaction;
Pharmacodynamic interaction;
Isobolographic analysis

**Abstract** The aim of this study was the isobolographic evaluation of interactions between losigamone (LSG), valproate (VPA), carbamazepine (CBZ), phenytoin (PHT), and phenobarbital (PB) in the maximal electroshock (MES) test in mice. Electroconvulsions were produced by means of an alternating current (ear-clip electrodes, 0.2-s stimulus duration, and tonic hindlimb extension taken as the endpoint). Adverse effects were evaluated in the chimney test (motor coordination) and the passive avoidance task (long-term memory). Brain concentrations of antiepileptic drugs (AEDs) were measured by immunofluorescence or high-performance liquid chromatography. Isobolographic analysis indicated synergistic interactions between LSG and VPA. For example, in the proportion of 1:1 the theoretically calculated 50% effective dose for additivity ($ED_{50add}$) was 138 mg/kg, while the experimentally derived $ED_{50}$ for the mixture ($ED_{50mix}$) was 85.2 mg/kg. The difference was significant at $p<0.001$. LSG combined with CBZ or PHT showed additivity, whereas the combinations of LSG with PB were either additive, for the fixed ratios of 1:3 and 1:1, or antagonistic for the ratio of 3:1 ($ED_{50add}$ = 18.4 mg/kg versus $ED_{50mix}$ = 26.7 mg/kg, $p<0.05$). Impairment of long-term memory was noted only in the case of VPA given at its $ED_{50}$, however this AED did not affect motor performance. LSG, CBZ, PHT and PB (applied at their $ED_{50}$ values) and co-administration of LSG with conventional AEDs (including VPA) impaired neither motor performance nor long-term memory. LSG did not affect the brain concentration of VPA or PB, but significantly elevated the brain concentrations of CBZ and PHT. In contrast, VPA, CBZ and PHT significantly increased the brain concentration of LSG, indicating a pharmacokinetic contribution to the observed pharmacodynamic interactions. Although LSG

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doi:10.1016/j.euroneuro.2006.02.005

exhibited some favorable pharmacodynamic interactions with various AEDs, these were complicated by pharmacokinetic interactions and emphasize the importance of measuring AED concentrations in studies designed to identify desirable AED combinations.


## 1. Introduction

Losigamone [LSG; AO-33; ADD137022; (±)-5(R,S)-α(S,R)-5-(2-chlorophenylhydroxymethyl)-4-methoxy-2(5H)-furanone], a novel antiepileptic drug, contains two asymmetric carbons and is a racemic mixture of two enantiomers, AO-242 [(+)-5(*R*)-α(*S*)-5-(2-chlorophenylhydroxymethyl)-4-methoxy-2(5H)-furanone] and AO-294 [(−)-5(*S*)-α(*R*)-5-(2-chlorophenylhydroxymethyl)-4-methoxy-2(5H)-furanone]. Experimental evidence indicates that LSG in rodents can inhibit the tonic hindlimb extension in the maximal electroshock (MES) test or that produced by various chemical convulsants, including pentylenetetrazole (PTZ), bicuculline, nicotine and 4-aminopyridine (Bialer et al., 1999). The drug also proved effective in antagonizing clonic seizures induced by PTZ, picrotoxin (PTX) and bicuculline (Bialer et al., 1999), and is effective against cocaine-induced convulsions in mice (Gasior et al., 1999), and audiogenic seizures in rats, gerbils (Bialer et al., 1999) and DBA/2 mice (Jones and Davies, 1999).

Electrophysiological and biochemical studies in vivo and in vitro have demonstrated that LSG possesses multiple mechanisms of action, which might account largely for its anticonvulsant activity. In rat hippocampal slices, LSG has been shown to reduce the frequency of spontaneous or stimulus-induced epileptiform discharges in the presence of PTX, low calcium ($Ca^{2+}$) or low magnesium ($Mg^{2+}$) artificial cerebrospinal fluid (Kohr and Heinemann, 1990a; Leschinger et al., 1993) or perfusion with high potassium ($K^+$), low $Mg^{2+}$ and low $Ca^{2+}$ fluid (Kohr and Heinemann, 1990a,b; Zhang et al., 1992). LSG may decrease neuronal excitability by a non-synaptic or direct membrane action, possibly involving inhibition of sodium ($Na^+$) or $Ca^{2+}$ influx or activation of $K^+$ efflux (Bialer et al., 1999). Moreover, the drug inhibited spontaneous depolarization of neurons, afterdischarges and *N*-methyl-D-aspartate (NMDA)-induced depolarization (Srinivasan et al., 1997). The NMDA receptor antagonism and inhibition of excitatory amino acid release may be considered as contributing factors to the anticonvulsant effect of LSG (Srinivasan et al., 1997). Complementary to these findings is an observation suggesting the LSG-induced stimulation of the γ-aminobutyric acid ($GABA_A$) receptor-mediated $Cl^-$ current (Dimpfel et al., 1995). This is supported by the ability of LSG to enhance $Cl^-$ uptake in mouse spinal cord neurons in the absence of GABA and to potentiate the effects of GABA, which in turn, was antagonized by tetrodotoxin and bicuculline (Dimpfel et al., 1995). It has been found that the drug does not affect specific GABA, flunitrazepam or *t*-butyl-bicyclophosphorothionate binding sites within the $GABA_A$ receptor complex (Willmore, 2001).

A number of conventional and newer AEDs, including phenytoin (PHT), carbamazepine (CBZ), valproate (VPA), lamotrigine or topiramate, inhibit $Na^+$ channels in mixed voltage- and activity-dependent manner (Ragsdale and Avoli, 1998). Furthermore, the $Na^+$ channels regulating action potential threshold are of persistent nature, while those responsible for action potential generation are characterized by transient openings (Gebhardt et al., 2001). In cultured hippocampal neurons (Gebhardt et al., 2001), the effects of LSG on the persistent $Na^+$ current ($I_{NaP}$) seemed to be similar to those of PHT (Lampl et al., 1998) and VPA (Taverna et al., 1998). However, both conventional AEDs showed additional use-dependent action on fast inactivating $Na^+$ currents ($I_{NaF}$) (Lampl et al., 1998; Taverna et al., 1998; Gebhardt et al., 2001). In contrast, LSG did not affect the fast $Na^+$ currents (Gebhardt et al., 2001).

In addition to its anticonvulsant activity, LSG possesses anxiolytic, antidepressant and memory enhancing activity in animal models (Bialer et al., 1999). In clinical trials, the drug was effective in the treatment of highly refractory partial seizures (with or without secondary generalized seizures) (Noldner and Chatterjee, 1990; Bialer et al., 1999). Preliminary data investigating the therapeutic dose range and its safety profile were obtained in a series of nine patients using an open label add-on ascending dose design. Patients with uncontrolled partial seizures receiving PHT, carbamazepine (CBZ) or their combinations, were administered with LSG (600—2100 mg daily). The best-tolerated therapeutic dose was 1500 mg/day. The median seizure reduction was 39%. Three patients out of nine remained seizure-free for 2 years. The most frequent adverse effects were headache and dizziness, however, their incidence was similar to those patients treated only with PHT or CBZ (Morris et al., 1997). More recently, a double-blind placebo controlled randomized add-on study was performed in 27 centers which included 203 patients with partial epilepsy. LSG was administered at the dose of 1500 mg/day. The concomitant treatment comprised of 2—3 AEDs, which included PHT, CBZ, VPA and phenobarbital (PB) (Baulac and Klement, 2003). The median relative reduction of seizure frequency was 14.9% in the LSG group versus 6.7% for placebo; the proportion of patients experiencing 50% seizure reduction was 22.3% versus 14.6% for placebo. LSG was well tolerated and although ophthalmological disorders occurred in the losigamone treatment groups, visual field defects were not observed (Baulac and Klement, 2003).

The purpose of the present study was to investigate the pharmacodynamic interaction profiles between LSG and various conventional AEDs (CBZ, PB, PHT and VPA) in the mouse maximal electroshock seizure model. Furthermore the interaction profiles were characterized in terms of behavioral paradigms (e.g. motor performance and long-term memory) and pharmacokinetic variables by measurement of brain concentrations of the tested drugs.