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The interactions of atorvastatin and fluvastatin with carbamazepine, phenytoin and valproate in the mouse maximal electroshock seizure model

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ABSTRACT

The aim of this study was to determine the influence of acute (single) and chronic (once daily for 7 consecutive days) treatments with atorvastatin and fluvastatin on the anticonvulsant potential of three antiepileptic drugs: carbamazepine, phenytoin and valproate in the mouse maximal electroshock-induced seizure model. Additionally, the effects of acute and chronic administration of both statins on the adverse effect potential of three antiepileptic drugs were assessed in the chimney test (motor performance) and passive avoidance task (long-term memory). To evaluate the pharmacokinetic characteristics of interaction between antiepileptic drugs and statins, the total brain concentrations of antiepileptic drugs were estimated with the fluorescence polarization immunoassay technique. Results indicate that atorvastatin at doses up to 80 mg/kg in chronic experiment attenuated the anticonvulsant potential of carbamazepine by increasing its ED_{50} value against maximal electroconvulsions. Acute fluvastatin (80 mg/kg) enhanced the anticonvulsant potential of carbamazepine and valproate by decreasing their ED_{50} values. Acute fluvastatin (80 mg/kg) also markedly increased the total brain carbamazepine concentration by 61% in a pharmacokinetic reaction. Atorvastatin (acute and chronic) and fluvastatin (chronic) in combinations with valproate impaired long-term memory in mice. Both statins in combinations with all three antiepileptic drugs had no impact on their adverse effects in the chimney test. Based on this preclinical study, one can conclude that chronic administration of atorvastatin reduces the anticonvulsant action of carbamazepine and acute fluvastatin can enhance the anticonvulsant potency of the carbamazepine and valproate. The former interaction was pharmacokinetic in nature.

1. Introduction

Statins, [3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) inhibitors], are potent lipid lowering medications used for hypercholesterolemia and coronary heart disease which have been shown to be protective in non-cardiovascular disorders, including neurological conditions such as multiple sclerosis and spinal cord injury (Etminan et al., 2010; Park et al., 2004; Smaldone et al., 2009) and intracranial haemorrhage (Naval et al., 2009). Statins reduce stroke incidence (Bösel et al., 2005; Endres and Laufs, 2004) and may reduce the risk of Alzheimer's disease, as shown in experimental studies (Jick et al., 2000; Kwak et al., 2000; Sierra et al., 2011; Wolozin et al., 2000).

It is estimated that 50 million people worldwide suffer from epilepsy (Brodie et al., 1997; Etminan et al., 2010) which is being treated long-term, often life-long (Patsalos et al., 2002). With the increase in the aging population worldwide, the prevalence of epilepsy will also increase (Kwan and Brodie, 2000). Additionally, given the common use of polypharmacy in elderly patients with epilepsy (Gidal et al., 2009), there is an increased likelihood of their being on drugs such as statins and antiepileptic drugs extensively metabolised via intestinal and hepatic CYP3A4. Consequently, the reduction in either oral bioavailability or increased systemic clearance could result in the diminished efficacy of these medications. This suggests that there may be an inadequate therapeutic response to the medical therapy (Candrilli et al., 2010). The phenomenon of pharmacokinetic interactions has been described for classical antiepileptic drugs, such as phenytoin, carbamazepine, valproate and barbiturates; and it has long been recognised as a potential complication factor in the management of patients with epilepsy (Patsalos et al., 2002). Phenytoin and carbamazepine are inductors of the cytochrome P450 (CYP) enzyme and the UDP-glucuronyltransferase (UGT) families of enzymes in the liver, and may reduce the concentration of statins in plasma and subsequently their potency (Candrilli et al., 2010; Corsini et al., 1999). By contrast, valproate is a liver enzyme inhibitor; thus in theory it may enhance the statin effect. Drug–drug interactions may lead to

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adverse drug reactions that can be severe enough in clinical presentation to require hospitalization (Egger et al., 2003).

Cleary et al. (2004) noted that approximately 30% of acute seizures in the elderly present as status epilepticus, with a mortality rate of approximately 40% (Cleary et al., 2004). Given that chronic inflammation is one of the hallmarks of epilepsy (Vezzani and Granata, 2005), it has been suggested that statins may protect against epilepsy via their anti-inflammatory properties (Lee et al., 2008). However, it is likely that the protective effect of statins in epilepsy may be through their effect in reducing the risk of stroke (Goldstein et al., 2008) which may be a strong risk factor in provoking seizures in the elderly.

Possible interactions between statins and antiepileptic drugs are an important determinant of safety in the long-term therapy of hypercholesterolemia and epilepsy. In this experimental study, we aimed to investigate the effect of atorvastatin and fluvastatin on the anticonvulsant potential of three commonly-used antiepileptic drugs: carbamazepine, phenytoin and valproate.

2. Materials and methods

2.1. Animals

All experiments were performed on adult male Swiss mice weighing 20–25 g. The animals were kept in cages with unlimited access to food and tap water, under standardised housing conditions such as a natural light–dark cycle, temperature of 21 ± 1 °C, and a relative humidity of 55 ± 5%. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups comprising of eight mice. Each mouse was used only once and all tests were performed between 10.00 am and 2.00 pm. The experimental protocols and procedures were conducted in accordance with current European Community and Polish legislation on animal experimentation. Furthermore, they were approved by the Local Ethics Committee at the Medical University of Lublin and confirmed with the Guide for the Care and Use of Laboratory Animals (1996) and with approval from the Ethics Commission as compliant with Polish Law (1997).

2.2. Drugs

The following drugs were used: atorvastatin (Sortis, Parke-Davis, Warsaw, Poland); fluvastatin (Lescol, Novartis, Warsaw, Poland); carbamazepine (Amizepin, Polfa, Warsaw, Poland); phenytoin (Phenytoin, Polfa, Warsaw, Poland); valproate magnesium (Dipromal, ICN, Polfa, Rzeszow); Tween 80 (Sigma, St. Louis, MO, USA). Both statins (atorvastatin and fluvastatin), carbamazepine and phenytoin were brought into solutions suspended in a 1% aqueous solution of Tween 80, whereas valproate was dissolved in a sterile saline. All antiepileptic drugs were administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight. Fresh drug solutions were prepared on each day of experimentation. Statins were administered orally.

2.3. Treatment protocol

This study consisted of two experiments associated with acute (single) and chronic (once daily for 7 days) administration of statins.

In the acute experiment mice were fed with atorvastatin and fluvastatin suspended in 1% aqueous solution of Tween 80. Statins were administered at a dose of 80 mg/kg (i.e., approximately 1.6 mg per mouse) per os via tube, 2 h prior to the experiment. Subsequently, the animals received intraperitoneally (i.p.) an injection with carbamazepine (30 min, before the test), phenytoin (120 min, before the test) and valproate (30 min, before the test).

In the chronic experiment, the animals were fed once daily at 10 am. The mean amount of chow pellets consumed by each individual mouse had been previously measured. Both atorvastatin and fluvastatin (as a lyophilised powder) were added to the food so that each mouse received a statin dose of 80 mg/kg once daily in the morning for 7 consecutive days. On the 8th day, the antiepileptic drugs were injected i.p. as in the acute experiment.

Subsequently, the animals were challenged with either electroshock-induced seizures, the chimney test, a passive avoidance task or brain sampling. Control animals received their usual food without the statins at respective times. All treatments were started on day 1 at 10 am.

2.4. The electroconvulsive threshold and the maximal electroshock seizure test

Electroconvulsions were produced by means of an alternating current provided by a Hugo Sachs generator (Rodent Shocker, type 221, Freiburg, Germany). The current (50 Hz, 0.2 s, and maximum stimulation voltage of 500 V) was delivered via ear-clip electrodes. The electrical system of the stimulator was self-adjustable so that changes in impedance did not result in alterations to current intensity in any given experimental group (i.e. the system provides constant current stimulation). Tonic hind limb extension (the hind limbs of animals outstretched 180° to the plane of the body axis) was taken as the endpoint. In this test, two experimental models of maximal electroconvulsions were used: 1) the maximal electroshock seizure threshold test and 2) the maximal electroshock seizure test.

To evaluate the threshold for maximal electroconvulsions, at least 4 groups of mice (8 animals in each group) were challenged with electroshocks of various intensities to yield 20–25%, 30–50%, 5–70%, and 70–90% of animals with seizures. Then, a current intensity–response relationship curve was created according to the log-probit method by Litchfield and Wilcoxon (1949), from which the median current strength (CS_{50} in mA) was calculated (Litchfield and Wilcoxon, 1949). The CS_{50} value represents the current intensity required to induce tonic hind limb extension in 50% of the challenged mice. After administration of both statins: atorvastatin and fluvastatin (either singly or chronically for 7 days) to four groups of animals, the mice were subjected to electroconvulsions (each group with a constant current intensity). The threshold for maximal electroconvulsions was recorded for two different doses of atorvastatin and fluvastatin, respectively 20 mg and 80 mg/kg. Subsequently, the percentage of increased CS_{50} values for animals injected with increasing doses of both statins over the control (vehicle-treated animals) was calculated. The procedure was described in our earlier study (Luszczki et al., 2007).

The protective potency of the various classical antiepileptic drugs (carbamazepine, phenytoin and valproate) was determined as their median effective doses (ED_{50} values in mg/kg) against maximal electroshock-induced seizures with a fixed current intensity of 25 mA. The animals were administered with different drug doses to obtain a variable percentage of protection against maximal electroshock seizures, allowing the construction of a dose–response relationship curve for each antiepileptic drug administered alone, according to Litchfield and Wilcoxon (1949). Each ED_{50} value represents the dose of a classical antiepileptic drug required to protect 50% of the animals tested against maximal electroshock seizures. Similarly, the anticonvulsant potency of a mixture of an antiepileptic drug with a statin was evaluated and expressed as ED_{50} corresponding to the dose of an antiepileptic drug necessary to protect 50% of mice against tonic hind limb extension in the maximal electroshock seizure test. In the present study, carbamazepine was administered at doses ranging between 4 and 12 mg/kg, phenytoin at doses ranging between 6 and 14 mg/kg and valproate at doses ranging between 175 and 300 mg/kg.

2.5. The chimney test

The chimney test was used to quantify the adverse effect of classical antiepileptic drugs administered alone, and in combination with atorvastatin and fluvastatin (at 80 mg/kg), respectively, on motor

performance in mice (Boissier et al., 1960). In this experiment, the animals had to climb backwards up a plastic transparent tube (3 cm inner diameter, 25 cm length), and motor performance impairment was indicated by the inability of the mice to climb backwards up the plastic transparent tube within 60 s. The adverse effect potential of classical antiepileptic drugs administered alone and in combination with statins were determined as their median toxic doses (TD_{50} values, in mg/kg; ± S.E.M.), representing the doses of antiepileptic drugs, necessary to impair motor coordination in 50% of the tested animals. The animals were administered with different drug doses to obtain a variable percentage of impairment of motor coordination in mice, allowing the construction of a dose–response relationship curve for each antiepileptic drug administered alone, according to Litchfield and Wilcoxon (1949). This experimental procedure has been described in detail in our study (Luszczki et al., 2007). Carbamazepine and phenytoin in the chimney test were administered i.p. at doses ranging between 70 and 100 mg/kg, whereas valproate was administered i.p. at doses between 350 and 600 mg/kg.

2.6. The light–dark, step-through passive avoidance test

Each animal was administered an antiepileptic drug either singly (at ED_{50} values from the maximal electroshock-induced seizure test) or with a combination of the statin. The time gap between the drug administration and the commencement of the training session was identical to that for electroconvulsions (carbamazepine and valproate — 30 min and phenytoin — 120 min prior to the test). Subsequently, animals were placed in an illuminated box (10×13×15 cm) connected to a larger dark box (25×20×15 cm) equipped with an electric grid floor. The entry of the animals into the dark box was punished by an adequate electric footshock (0.6 mA for 2 s). The animals, which did not enter the dark compartment within 60 s, were excluded from subsequent experiments. On the following day (24 h later), the pretrained animals were placed once more into the illuminated box and observed for up to 180 s. Mice which avoided the dark compartment for 180 s were regarded as remembering the task. The time that mice took to enter the dark box was noted and the median retention times with 25th and 75th percentiles were calculated. Overall, the test provides information on the ability to acquire the task (learning) and to recall the task (retrieval). Hence, it may be regarded as a measure of long-term memory (Venault et al., 1986).

2.7. The measurement of total brain antiepileptic drug concentrations

The mice were administered one of the classical antiepileptic drugs and vehicle (at the ED_{50} values from the maximal electroshock-induced seizure test) or with the antiepileptic drugs plus a respective statin. The animals were killed by decapitation at times chosen to coincide with those scheduled for the maximal electroshock-induced seizure test. Brains were removed from skulls, weighed, and homogenised using an Abbott buffer (2:1 vol/weight) in an Ultra-Turrax T8 homogeniser (Stauffen, Germany) at a temperature of 4 °C. The homogenates were centrifuged at 10,000 *g* for 10 min. The supernatant samples (50 μl) were analysed by fluorescence polarization immunoassay (FPIA) for carbamazepine, phenytoin and valproate content, using a TDx analyzer and reagents exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). All antiepileptic drug concentrations are expressed in μg/ml of brain supernatants as the means ± S.D. of at least eight separate brain measurements.

2.8. Statistics

CS_{50}, ED_{50} and TD_{50} values ± S.E.M. were estimated using computer log-probit analysis according to Litchfield and Wilcoxon (1949). One-way ANOVA followed by the post hoc Tukey–Kramer test for multiple comparisons, was applied for the statistical evaluation of CS_{50} and ED_{50} values. The results from the step-through passive avoidance task were statistically evaluated using Kruskal–Wallis nonparametric ANOVA followed by the post-hoc Dunn's test. Total brain concentrations of antiepileptic drugs were statistically compared using the unpaired Student's *t*-test. An index of probability less than 0.05 ($p<0.05$) was considered as significant.

3. Results

3.1. The effects of acute and chronic administration of atorvastatin and fluvastatin on the seizure threshold and the anticonvulsant potency of carbamazepine, phenytoin and valproate against maximal electroshock-induced seizures

In an acute experiment, atorvastatin and fluvastatin at the doses of 20 and 80 mg/kg respectively had no effect on the electroconvulsive threshold. In a chronic administration over 7 days, atorvastatin and fluvastatin (20 and 80 mg/kg) did not influence the seizure threshold either. Therefore, for the combination with antiepileptic drugs statins were used at doses not affecting the electroconvulsive threshold.

3.2. The effects of atorvastatin and fluvastatin on the protective action of classical antiepileptic drugs in the mouse maximal electroshock-induced seizure model

The ED_{50} values of carbamazepine, phenytoin and valproate against the maximal electroshock-induced seizures are shown in Tables 1 and 2.

Atorvastatin administered chronically for 7 days at 80 mg/kg significantly reduced the anticonvulsant effect of carbamazepine against maximal electroshock-induced seizures by increasing its ED_{50} value from 8.8 to 12.5 mg/kg (Table 1).

Conversely, fluvastatin in acute doses of 10, 20, 40 and 80 mg/kg increased the anticonvulsant potency of carbamazepine against maximal electroshock-induced seizures by reducing its ED_{50} values from 11.4 to 5.9, 5.6, 5.0 and 5.6 mg/kg, respectively (Table 2). However,

Table 1
The effects of acute and chronic administration of atorvastatin on the anticonvulsant potency of the classical antiepileptic drugs: carbamazepine, phenytoin and valproate against maximal electroshock-induced seizures in mice.

Treatment (mg/kg)	ED_{50} (mg/kg)	n
Acute		
Carbamazepine + vehicle	11.4 ± 0.83	16
Carbamazepine + atorvastatin (80)	9.4 ± 1.12	16
Phenytoin + vehicle	10.5 ± 0.96	8
Phenytoin + atorvastatin (80)	9.4 ± 1.12	16
Valproate + vehicle	237.5 ± 18.91	24
Valproate + atorvastatin (80)	241.2 ± 21.03	16
Chronic		
Carbamazepine + vehicle	8.8 ± 0.84	16
Carbamazepine + atorvastatin (40)	10.8 ± 1.28	16
Carbamazepine + atorvastatin (80)	12.5 ± 0.88*	16
F (2;45) = 3.297; P = 0.046		
Phenytoin + vehicle	8.8 ± 0.75	16
Phenytoin + atorvastatin (80)	9.0 ± 1.00	16
Valproate + vehicle	237.5 ± 18.91	24
Valproate + atorvastatin (80)	269.6 ± 31.52	24

Results are presented as median–effective doses (ED_{50} in mg/kg; ± S.E.M.) required to protect 50% of animals tested against maximal electroshock-induced seizures. The ED_{50} values were calculated by the use of the log-probit method. Statistical analysis of data was performed either with the log-probit method (for single comparisons) or with one-way ANOVA followed by the post-hoc Tukey–Kramer test (for multiple comparisons). All antiepileptic drugs were administered i.p.; carbamazepine and valproate — 30 min and phenytoin — 120 min prior to the maximal electroshock-induced seizures. n — total number of animals used at those doses whose anticonvulsant effects ranged between 4 and 6 probits. F — F-statistics from one-way ANOVA; P — probability from one-way ANOVA.

* $P<0.01$ vs. the respective control group (an antiepileptic drug alone).

Table 2
The effects of acute and chronic administration of fluvastatin on the anticonvulsant potency of the classical antiepileptic drugs: carbamazepine, phenytoin and valproate in the maximal electroshock-induced seizures in mice.

Treatment (mg/kg)	ED_{50} (mg/kg)	n
Acute		
Carbamazepine + vehicle	11.4 ± 0.77	16
Carbamazepine + fluvastatin (5)	10.2 ± 0.80	16
Carbamazepine + fluvastatin (10)	5.9 ± 0.77**	8
Carbamazepine + fluvastatin (20)	5.6 ± 0.62**	8
Carbamazepine + fluvastatin (40)	5.0 ± 0.70**	24
Carbamazepine + fluvastatin (80)	5.6 ± 1.08**	24
F (5;90) = 9.109; P<0.0001		
Valproate + vehicle	237.5 ± 18.07	24
Valproate + fluvastatin (20)	226.8 ± 24.75	16
Valproate + fluvastatin (40)	136.2 ± 19.50**	16
Valproate + fluvastatin (80)	181.4 ± 12.71*	24
F (3;76) = 5.910; P = 0.0011		
Phenytoin + vehicle	10.5 ± 0.90	16
Phenytoin + fluvastatin (80)	9.6 ± 0.70	16
Chronic		
Carbamazepine + vehicle	8.8 ± 0.84	16
Carbamazepine + fluvastatin (80)	10.2 ± 1.00	8
Phenytoin + vehicle	8.8 ± 0.84	16
Phenytoin + fluvastatin (80)	9.0 ± 1.21	8
Valproate + vehicle	237.5 ± 18.07	24
Valproate + fluvastatin (80)	273.3 ± 25.99	16

Results are presented as median effective doses (ED_{50} in mg/kg; ± S.E.M.) required to protect 50% of animals tested against maximal electroshock-induced seizures. The ED_{50} values were calculated by the use of log-probit method. Statistical analysis of data was performed either with the log-probit method (for single comparisons) or with one-way ANOVA followed by the post-hoc Tukey–Kramer test (for multiple comparisons). n — the total number of animals used at those doses whose anticonvulsant effects ranged between 4 and 6 probits. F — F-statistics from one-way ANOVA; P — probability from one-way ANOVA. Refer also to footnotes of Table 1.

* P<0.01 vs. the respective control group (antiepileptic drug alone).

** P<0.001 vs. the respective control group (antiepileptic drug alone).

acute fluvastatin at 40 and 80 mg/kg enhanced the anticonvulsant potency of valproate against maximal electroshock-induced seizures by reducing its ED_{50} values from 237.5 to 181.4 and 136.2 mg/kg, respectively (Table 2).

Atorvastatin at a dose of 80 mg/kg administered for 7 days had no significant effect on the antielectroshock action of valproate (Table 1). Both statins applied acutely or chronically were ineffective in modifying the protective potency of phenytoin against maximal electroshock-induced seizures in mice (Tables 1 and 2).

3.3. The effects of atorvastatin and fluvastatin in combination with classical antiepileptic drugs on long-term memory and motor performance

Neither atorvastatin nor fluvastatin (both at 80 mg/kg), given acutely or chronically, impaired the performance of mice in the passive avoidance task (Tables 3 and 4) or chimney test.

By contrast, valproate administered alone at a dose of 241.2 mg/kg significantly impaired long-term memory in mice subjected to the step-through passive avoidance test (Table 3). In acute and chronic experiments, when atorvastatin (80 mg/kg) was administered in combination with valproate at doses corresponding to their ED_{50} value, long-term memory determined in the passive avoidance test, was significantly impaired compared to median retention time, but it was not significant as compared to valproate alone (Table 3). Likewise, in chronic experiment fluvastatin (80 mg/kg) administered in combination with valproate markedly reduced long-term memory in mice when compared to the median retention time of 180 s, which was not significantly different from the retention time for valproate alone (Table 4). Neither atorvastatin nor fluvastatin (80 mg/kg) in combination with valproate altered motor performance in mice.

Table 3
The effects of atorvastatin and its combinations with classical antiepileptic drugs: carbamazepine, phenytoin and valproate on long-term memory in the passive avoidance task in mice.

Treatment (mg/kg)	Retention time (s)
Acute	
Vehicle	180 (180–180)
Atorvastatin (80) + vehicle	180 (101.5–180)
Carbamazepine (9.4) + vehicle	180 (180–180)
Carbamazepine (9.4) + atorvastatin (80)	180 (180–180)
Phenytoin (9.4) + vehicle	180 (165–180)
Phenytoin (9.4) + atorvastatin (80)	180 (180–180)
Valproate (241.2) + vehicle	80.5 (33.5–180)*
Valproate (241.2) + atorvastatin (80)	82 (53.5–168)*
Chronic	
Vehicle	180 (180–180)
Atorvastatin (80) + vehicle	180 (85–180)
Carbamazepine (12.5) + vehicle	180 (180–180)
Carbamazepine (12.5) + atorvastatin (80)	180 (74–180)
Phenytoin (9.0) + vehicle	180 (158.5–180)
Phenytoin (9.0) + atorvastatin (80)	180 (150–180)
Valproate (269.6) + vehicle	99 (23–180)*
Valproate (269.6) + atorvastatin (80)	123 (33–179)*

Results are presented as median retention times (in seconds; with 25th and 75th percentiles in parentheses), assessing long-term memory in mice. Each experimental group consisted of 8 mice. Statistical analysis of data was performed with nonparametric Kruskal–Wallis ANOVA followed by the post-hoc Dunn's test. All three antiepileptic drugs were administered i.p. at times scheduled from the maximal electroshock seizure test and at doses corresponding to their ED_{50} values against maximal electroconvulsions. Refer also to the footnotes of Table 1.

* P<0.05 vs. the respective control group (antiepileptic drug administered alone).

On the contrary, atorvastatin and fluvastatin (80 mg/kg), neither in acute nor chronic experiments, when administered in combination with carbamazepine or phenytoin affected the long-term memory as assessed in the passive avoidance test, or motor performance as determined in the chimney test.

Table 4
The effects of fluvastatin and its combinations with classical antiepileptic drugs: carbamazepine, phenytoin and valproate on long-term memory in mice in the passive avoidance task.

Treatment (mg/kg)	Retention time (s)
Acute	
Control (vehicle-treated animals)	180 (180–180)
Fluvastatin (80) + vehicle	180 (180–180)
Carbamazepine (5.6) + vehicle	180 (180–180)
Carbamazepine (5.6) + fluvastatin (80)	180 (110–180)
Phenytoin (9.6) + vehicle	180 (165–180)
Phenytoin (9.6) + fluvastatin (80)	180 (180–180)
Valproate (181.4) + vehicle	180 (180–180)
Valproate (181.4) + fluvastatin (80)	180 (180–180)
Chronic	
Control (vehicle-treated animals)	180 (180–180)
Fluvastatin (80) + vehicle	180 (180–180)
Carbamazepine (10.5) + vehicle	180 (180–180)
Carbamazepine (10.5) + fluvastatin (80)	180 (74–180)
Phenytoin (9.0) + vehicle	180 (165–180)
Phenytoin (9.0) + fluvastatin (80)	180 (165–180)
Valproate (273.3) + vehicle	88.5 (55.5–151)*
Valproate (273.3) + fluvastatin (80)	50 (16–180)*

Results are presented as median retention times (in seconds; with 25th and 75th percentiles in parentheses) from the passive avoidance task, assessing long-term memory in mice. Each experimental group consisted of 8 mice. Statistical analysis of data was performed with nonparametric Kruskal–Wallis ANOVA followed by the post-hoc Dunn's test. All three antiepileptic drugs: carbamazepine, valproate and phenytoin were administered i.p. at times scheduled for the maximal electroshock seizure test and at doses corresponding to their ED_{50} values against maximal electroconvulsions. Refer also to the footnotes of Table 1.

* P<0.05 vs. the respective control group (antiepileptic drug administered alone).

3.4. The influence of statins on total antiepileptic drug brain concentrations

Pharmacokinetic verifications were only carried out in cases of significant modifications by statins of the anticonvulsant ED_{50} values of the antiepileptic drugs. Acute fluvastatin (80 mg/kg) elevated by 61% the total brain concentration of carbamazepine (5.6 mg/kg) from 0.53 to 0.85 μg/ml ($P<0.01$; Table 5). On the other hand, acute fluvastatin remained ineffective on the total brain concentration of valproate (181.4 mg/kg; Table 5). Chronic atorvastatin (80 mg/kg) did not significantly modify the total brain concentration of carbamazepine (12.5 mg/kg; Table 5).

4. Discussion

The results of this study show for the first time that atorvastatin and fluvastatin are able to modify the anticonvulsant potency of classical antiepileptic drugs. Most statins and classical antiepileptic drugs undergo a similar metabolism pathway that could explain potential pharmacokinetic interactions. Drug interactions involving statins may have a pharmacodynamic or pharmacokinetic basis, or both (Williams and Feely, 2002).

The positive interaction of fluvastatin with carbamazepine has been associated with a considerable increase in the total brain concentration of this antiepileptic drug. The underlying mechanism for this interaction is still unclear. It seems less likely to be related to metabolism, since carbamazepine is a substrate of CYP3A4 and fluvastatin is metabolised mainly by CYP2C9. The most plausible mechanism is pharmacokinetic interactions; however, pharmacodynamic interactions cannot be excluded either. Although fluvastatin is a potent inhibitor of CYP2C9 in the liver, to a less extent by CYP3A4 and CYP2D6 (Bellosta et al., 2004; Scripture and Pieper, 2001), this effect is limited as a result of the rapid systemic clearance of the statin, and will only affect the metabolism of co-administered compounds during the first-pass (Williams and Feely, 2002). This makes the drug less prone to drug–drug interactions (Neuvonen et al., 2008). In addition, fluvastatin demonstrates inhibitory effects on this isoenzyme in vitro and in vivo. In the human liver microsomes, fluvastatin inhibits the hydroxylation of CYP2C9 substrates and produces a slight reduction in the clearance of glyburide, tolbutamide and diclofenac (Appel et al., 1995; Scripture and Pieper, 2001; Shitara and Sugiyama, 2006). However, it has no effects on their hypoglycaemic action, which suggests that the pharmacokinetic changes are not great enough to influence their pharmacological effects (Appel et al., 1995). The statin reaches its maximum concentration after 0.5 to 1.5 h similarly to carbamazepine (after 0.5 h). Noteworthily, fluvastatin has showed low permeability in in vivo models with brain perfusion (Saheki et al., 1994).

The combined treatment of chronic atorvastatin with carbamazepine merits special attention, as the anticonvulsant potency of this antiepileptic drug has been distinctly reduced. Moreover, the total brain concentration of carbamazepine has not been significantly affected by the statin, which could be explained via the pharmacodynamic mechanism. Atorvastatin, similarly to carbamazepine, is biotransformed via intestinal and hepatic CYP 3A4 and highly extracted by the liver (Bellosta et al., 2004; Shitara and Sugiyama, 2006). After it is absorbed from the gastrointestinal tract, peak concentration occurs within 1 to 2 h (Williams and Feely, 2002). It is noteworthy that the reported half-life of statins does not correspond to the duration of their pharmacodynamic effect (approximately 24 h). However, due to its long elimination half-life (14 h), and to the presence of detectable plasma levels of active metabolites for a prolonged period of time (>24 h) (Posvar et al., 1996), atorvastatin can accumulate in the plasma, achieving a steady-state drug concentration after multiple doses (Cilia et al., 1996; Corsini et al., 1999). There are limited data on the interactions of statins and antiepileptic drugs. Although pharmacokinetic interactions have been clearly shown in experimental and clinical studies, there is still a lack of data verifying whether such interactions are clinically significant. Carbamazepine has been shown to induce its own metabolism via CYP3A4 (Scheyer et al., 1994) and exhibit the ability to reduce the plasma concentration of simvastatin by enzyme induction (Ucar et al., 2004). In the case report it has been the antiepileptic drug that affected the statin's lipid lowering potential and reduced the peak concentration of simvastatin and simvastatin acid by 68% (Ucar et al., 2004).

Phenytoin and valproate are protein bound and they compete for protein binding with other drugs. This can result in the displacement of antiepileptic drugs from their plasma protein–drug complex, resulting in increases in the free fraction of the previously protein-bound antiepileptic drug (Patsalos et al., 2002). As phenytoin is 80% metabolised by isoenzyme CYP2C9, it makes it susceptible to problematic interactions with other CYP2C9 substrates such as fluvastatin. Although in the literature phenytoin is associated with more drug interactions than carbamazepine or valproate (Patsalos et al., 2002), in our study its potency has not been significantly altered by statins. It should be borne in mind that in our study we have examined the influence of statins on the antiepileptic drugs and not the opposite. The interactions described in case reports highlight the effect of antiepileptic drugs on statin concentrations. Phenytoin has been identified as decreasing statins' concentrations and affecting their potency. One of case reports has indicated that phenytoin can alter the efficacy of both atorvastatin and simvastatin, and to optimise the lipid profile the atorvastatin dose has to be increased (Murphy and Dominiczak, 1998). Although the serum cholesterol level in patients with hypercholesterolemia taking simvastatin increases after taking phenytoin, there is no evidence that its plasma concentration has been altered. This may be explained by the induction of CYP3A4, leading to an increase in the clearance of simvastatin. The concentrations of statins, particularly simvastatin and atorvastatin, decrease after they have been combined with barbiturates, carbamazepine and phenytoin (Miller et al., 2008; Ratz Bravo et al., 2005). It might have potentially resulted in the loss of statins' clinical effect; however, in view of the rarity of such interactions (1.4%), their clinical relevance is unclear (Ratz Bravo et al., 2005). Another clinical vignette on the interaction of the statin and phenytoin (Tan et al., 2007) has suggested P-glycoprotein, an ATP-dependent drug transport protein in the intestinal plasma membrane, as a source of interaction (Corsini et al., 1999; Holtzman et al., 2006). Phenytoin is known to be an inducer of P-glycoprotein and potentially can increase statin concentration (Tan et al., 2007). The results of in vitro models have shown that lovastatin, simvastatin and atorvastatin are inhibitors

Table 5
The effects of atorvastatin and fluvastatin on total brain concentrations of antiepileptic drugs.

Treatment (mg/kg)	Brain concentration (μg/ml)	
Acute experiment		
Carbamazepine (5.6) + vehicle	0.53 ± 0.18	
Carbamazepine (5.6) + fluvastatin (80)	0.85 ± 0.16*	↑61%
Valproate (181.4) + vehicle	13.84 ± 2.92	
Valproate (181.4) + fluvastatin (80)	13.00 ± 2.25	
Chronic experiment		
Carbamazepine (12.5) + vehicle	1.94 ± 0.37	
Carbamazepine (12.5) + atorvastatin (80)	1.74 ± 0.53	

Data are presented as means ± S.E.M. of at least 8 separate measurements. Total brain concentrations of two antiepileptic drugs (carbamazepine and valproate) were performed with the fluorescence polarisation immunoassay technique and were determined in μg/ml of brain supernatants. Data were statistically verified by using the unpaired Student's *t*-test. All drugs were administered i.p. at doses corresponding to their ED_{50} values from the maximal electroshock-induced seizures. Refer also to footnotes of Table 1.

↑ — increase in total brain carbamazepine concentration in comparison with the control group.

* $P<0.01$ vs. Carbamazepine + vehicle.

for P-glycoprotein and may be substrates for this transporter as well. Pravastatin and fluvastatin however demonstrate no significant inhibition of P-glycoprotein (Holtzman et al., 2006).

Valproate, though a CYP enzymes inhibitor, being capable of reducing the rate of metabolism of the co-administered drug, has not been presented yet as eliciting any interactions with statins. However, in our study we demonstrate for the first time that the positive interaction of acute fluvastatin with valproate is of a pharmacodynamic nature. It needs to be highlighted that both valproate and fluvastatin are CYP2C9 substrates and compete for the same binding sites in the enzyme, so a pharmacokinetic interaction could not be excluded. It is noteworthy that valproate is ~80% metabolised in the liver via glucuronidation and β oxidation (Patsalos et al., 2002) similarly to atorvastatin. However, in our study it is fluvastatin that enhanced the valproate's anticonvulsant potential.

So far there have been very few cohort studies confirming the interactions between statins and antiepileptic drugs. Candrilli et al. (2010) in their retrospective study have shown that a greater proportion of patients receiving enzyme-inducing antiepileptic drugs along with atorvastatin or simvastatin have a stroke during the follow-up period when compared with the non-enzyme-inducing antiepileptic drugs group. This could have important implications on cardiovascular and cerebrovascular outcomes (Candrilli et al., 2010).

In summary, the antagonistic interaction between chronic atorvastatin and carbamazepine in the test of maximal electroshock-induced convulsions in mice has been documented. In the cases of phenytoin and valproate, their interactions with atorvastatin administered chronically are neutral. Chronic fluvastatin has not modified the anticonvulsant potency of the tested antiepileptic drugs. In the light of the above results, one can assume that chronic therapy with atorvastatin may reduce the antiseizure action of carbamazepine in epileptic patients. The acute experiments may indicate that initial transient positive interactions between valproate and fluvastatin may be encountered in clinical conditions. As for carbamazepine, its combination with acute fluvastatin has involved a pharmacokinetic interaction. To the extent that the experimental results may be transferred to the treatment of human epilepsy, the present data point to generally safe combinations of statins with antiepileptic drugs. The impairment of long-term memory seen in some combinations of statins with valproate may be entirely ascribed to this antiepileptic drug alone.

Conflicts of interest

Professor S.J. Czuczwar has received support from UCB Pharma and Sanofi-Aventis as a speaker. The remaining authors have no conflicts of interest to disclose.

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References

Appel, S., Rufenacht, T., Kalafsky, G., Tetzloff, W., Kallay, Z., Hitzenberger, G., Kutz, K., 1995. Lack of interaction between fluvastatin and oral hypoglycemic agents in healthy subjects and in patients with non-insulin-dependent diabetes mellitus. Am. J. Cardiol. 76, 29A–32A.

Bellosta, S., Paoletti, R., Corsini, A., 2004. Safety of statins: focus on clinical pharmacokinetics and drug interactions. Circulation 109, III-50–III-57.

Boissier, J.R., Tardy, J., Diverres, J.C., 1960. Une nouvelle méthode simple pour explorer l'action tranquilisante: Le test de la cheminée. Med. Exp. (Basel) 3, 81–84.

Bösel, J., Gandor, F., Harms, C., Synowitz, M., Harms, U., Djoufack, P.C., Megow, D., Dirnagl, U., Hortnagl, H., Fink, K.B., Endres, M., 2005. Neuroprotective effects of atorvastatin against glutamate-induced excitotoxicity in primary cortical neurones. J. Neurochem. 92, 1386–1398.

Brodie, M.J., Shorvon, S.D., Canger, R., Halasz, P., Johannessen, S., Thompson, P., Wieser, H.G., Wolf, P., 1997. Commission on European affairs: appropriate standards of epilepsy care across Europe. Epilepsia 38, 1245–1250.

Candrilli, S.D., Manjunath, R., Davis, K.L., Gidal, B.E., 2010. The association between antiepileptic drugs and HMG-CoA reductase inhibitor co-medication and cholesterol management in patients with epilepsy. Epilepsy Res. 91, 260–266.

Cilia, D.D., Whitfiled, L.R., Gibson, D.M., Sedman, A.J., Posvar, E.L., 1996. Multiple-dose pharmacokinetics, pharmacodynamics, and safety of atorvastation, an inhibitor of HMG-CoA reductase, in healthy subjects. Clin. Pharmacol. Ther. 60, 687–695.

Cleary, P., Shorvon, S., Tallis, R., 2004. Late onset seizures as a predictor of subsequent stroke. Lancet 363, 1184–1186.

Corsini, A., Bellosta, S., Baetta, R., Fumagalli, R., Paoletti, R., Bernini, F., 1999. New insight into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol. Ther. 84, 413–428.

Egger, S.S., Drewe, J., Schlienger, R.G., 2003. Potential drug–drug interactions in the medication of medical patients at hospital discharge. Eur. J. Clin. Pharmacol. 58, 773–778.

Endres, M., Laufs, U., 2004. Effects of statins on endothelium and signalling mechanisms. Stroke 35, 2708–2711.

Etminan, M., Samii, A., Brophy, J.M., 2010. Statin use and risk of epilepsy. Neurology 75, 1496–1500.

Gidal, B.E., French, J.A., Grossman, P., LeTeuff, G., 2009. Assessment of potential drug interactions in patients with epilepsy. Impact of age and sex. Neurology 72, 419–425.

Goldstein, L.B., Amarenco, P., Lamonte, M., Gilbert, S., Messig, M., Callahan, A., Hennerici, M., Sillesen, H., Welch, K.M., Investigators, S.P.A.R.C.L., 2008. Relative effect of statin therapy on stroke and cardiovascular events in men and women: secondary analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels study. Stroke 39, 2444–2448.

Holtzman, C.W., Wiggins, B.S., Spinler, S.A., 2006. Role of p-glycoprotein in statin drug interactions. Pharmacotherapy 26, 1601–1607.

Jick, H., Zornberg, G.L., Jick, S.S., Seshadri, S., Drachman, D.A., 2000. Statins and the risk of dementia. Lancet 356, 1627–1631.

Kwak, B., Mulhaupt, F., Myit, S., Mach, F., 2000. Statins as a newly recognized type of immunomodulator. Nat. Med. 6, 1399–1402.

Kwan, P., Brodie, M.J., 2000. Early identification of refractory epilepsy. N. Engl. J. Med. 342, 314–319.

Lee, J.K., Won, J.S., Singh, A.K., Singh, I., 2008. Statin inhibits kainic acid-induced seizure and associated inflammation and hippocampal cell death. Neurosci. Lett. 440, 260–264.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. 96, 99–113.

Luszczki, J.J., Glowniak, K., Czuczwar, S.J., 2007. Imperatorin enhances the protective activity of conventional antiepileptic drugs against maximal electroshock-induced seizures in mice. Eur. J. Clin. Pharmacol. 574, 133–139.

Miller, J.M., Bullman, J.N., Alexander, S., VanLandingham, K.E., 2008. A prospective assessment of the effect of phenytoin on the pharmacokinetics of atorvastatin. Epilepsia 49, 444.

Murphy, M.J., Dominiczak, M.H., 1998. Efficacy of statin therapy: possible effect of phenytoin. Postgrad. Med. J. 75, 359–361.

Naval, N.S., Mirski, M.A., Carhuapoma, J.R., 2009. Impact of statins on validation of ICH mortality prediction models. Neurol. Res. 31, 425–429.

Neuvonen, P.J., Backman, J.T., Niemi, M., 2008. Pharmacokinetic comparison of the potential over-the-conter statins simvastatin, lovastatin, fluvastatin and pravastatin. Clin. Pharmacokinet. 47, 463–474.

Park, E., Velumian, A.A., Fehlings, A.G., 2004. The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J. Neurotrauma 21, 754–774.

Patsalos, P.N., Fröscher, W., Pisani, F., vanRijn, C.M., 2002. The importance of drug interactions in epilepsy therapy. Epilepsia 43, 365–385.

Posvar, E.L., Radulovic, L.L., Cilia, D.D., Whitfield, L.R., Dedman, A.J., 1996. Tolerance and pharmacokinetics of single-dose atorvastatin, a potential inhibitor of HMG-CoA reductase, in healthy subjects. J. Clin. Pharmacol. 36, 728–731.

Ratz Bravo, A.E., Tchambaz, L., Krahenbuhl-Mechler, A., Hess, L., Schlieger, R.G., Krahenbuhl, S., 2005. Prevalence of potentially severe drug–drug interactions in ambulatory patients with dyslipidaemia receiving HMG-CoA reductase inhibitor therapy. Drug Saf. 28, 263–275.

Saheki, A., Terasaki, T., Tamai, I., Tsuji, A., 1994. In vitro and in vivo blood brain barrier transport of 3-hydroxy-3-methylglutaryl coenzyme A (MG-CoA) reductase inhibitors. Pharm. Res. 11, 305–311.

Scheyer, R.D., Cramer, J.A., Mattson, R.H., 1994. A pharmacodynamic approach to the estimate of carbamazepine autoinduction. J. Pharm. Sci. 84, 491–494.

Scripture, C.D., Pieper, J.A., 2001. Clinical pharmacokinetics of fluvastatin. Clin. Pharmacokinet. 40, 263–281.

Shitara, Y., Sugiyama, Y., 2006. Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug–drug interactions and interindividual differences in transporter and metabolic enzyme functions. Pharmacol. Ther. 112, 71–105.

Sierra, S., Ramos, M.C., Molina, P., Vazquez, J.A., Burgos, J.S., 2011. Statins as neuroprotectants: A comparative in vivo study of lipophilicity, blood–brain-barrier penetration, lowering of brain cholesterol, and decrease of neuron cell death. J. Alzheimers Dis. 23, 307–318.

Smaldone, C., Brugaletta, S., Pazzano, V., Liuzzo, G., 2009. Immunomodulator activity of 3-hydroxy-3-methilglutaryl-CoA inhibitors. Cardiovasc. Hematol. Agents Med. Chem. 7, 279–294.

Tan, K.M., Kelly, J.G., McGarry, K., 2007. Statins and phenytoin interact—a case history. Br. J. Clin. Pharmacol. 65, 147–148.
Ucar, M., Neuvonen, M., Luurila, H., Dahlqvist, R., Neuvonen, P.J., Mjorndal, T., 2004. Carbamazepine markedly reduces serum concentrations of simvastatin and simvastatin acid. Eur. J. Clin. Pharmacol. 59, 879–882.
Venault, P., Chapouthier, G., deCarvalho, L.P., Simiand, J., Morre, M., Dodd, R.H., Rossier, J., 1986. Benzodiazepine impairs and beta-carboline enhances performance in learning and memory task. Nature 321, 864–866.
Vezzani, A., Granata, T., 2005. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia 46, 1724–1743.
Williams, D., Feely, J., 2002. Pharmacokinetic–pharmadynamic drug interactions with HMG-CoA reductase inhibitors. Clin. Pharmacokinet. 41, 343–370.
Wolozin, B., Kellman, W., Ruosseau, P., Celesia, G.G., Siegel, G., 2000. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Arch. Neurol. 57, 1439–1443.