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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