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Original article

Pharmacodynamic and pharmacokinetic interactions between simvastatin and diazepam in rats

Wojciech Słupski*, Małgorzata Trocha, Maria Rutkowska

Department of Pharmacology, Wroclaw Medical University, Wrocław, Poland

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ABSTRACT

Background: Statins and benzodiazepines are widely used drugs, especially in ischemic heart disease, where exacerbation caused by anxiety can even lead to cardiac death. There have not been any reports of statin drug interaction with anxiolytics so far, but it is possible that these drugs interact with each other. We examined the effect of chronic oral administration of simvastatin on the anxiolytic activity and pharmacokinetics of diazepam in rats.

Methods: Studies were conducted on male Wistar Han rats treated with simvastatin (2.5, 5, 10, 20 mg/kg) for 4–6 weeks, and/or diazepam (2.5, 5, 10 mg/kg) administered once on the day of the study. Evaluation of potential pharmacodynamic interaction was based on the behavioral tests: elevated plus maze (EPM) test and the Vogel conflict test (VCT). The assessment of the potential pharmacokinetic interaction was based on measurements of concentrations of diazepam and its metabolites in the blood of animals.

Results: Diazepam 5 and 10 mg/kg given together with simvastatin 10 and 20 mg/kg showed no anxiolytic effect in the EPM test. In the VCT diazepam combinations with simvastatin did not produce any anxiolytic effect either, with an exception of the co-administration of diazepam 10 mg/kg and simvastatin 10 mg/kg. Simvastatin (20 mg/kg) significantly reduced the area under curve (AUC) of diazepam by 51.6% and temazepam by 54.6%.

Conclusions: Abolition of diazepam anxiolytic effect during concomitant use of simvastatin is probably caused by diminished bioavailability of diazepam, although pharmacodynamic interaction between these drugs cannot be excluded.

Introduction

Anxiety is an important risk factor of cardiovascular diseases. It could be discussed in two ways: as a source of cardiovascular symptoms and as a consequence of cardiovascular diseases. Anxiety actually increases the activity of the sympathetic system and reduces tension of the parasympathetic system [1].

Benzodiazepines, one of the most important groups of anxiolytic drugs, are often used in cardiac patients [2]. Those drugs act as positive allosteric modulators of the type-A γ-amino-butyric acid receptors ($GABA_AR$s), facilitating the interaction between the receptor and its neurotransmitter, leading to a decreased neuronal activity. Diazepam, as a one of the most widely used drug in this group, exhibits anxiolytic effect by the interaction with the α2 subunit of $GABA_AR$s in the limbic system [3]. In the liver diazepam is metabolized to three main active metabolites: desmethyldiazepam, oxazepam and temazepam. The metabolism of diazepam depends on the species, age, sex, presence of liver diseases, genetic variability and the exposure to inducers or inhibitors of cytochrome P450 (CYP450) [4].

The 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase inhibitors, commonly known as statins, are the most potent and most widely used cholesterol lowering drugs. They inhibit the conversion of HMG-CoA to mevalonic acid, leading to reduced biosynthesis of cholesterol. They have also the following pleiotropic properties: improvement of the endothelial function, stabilization of atheromatous plaque, an increase of nitric oxide (NO) synthesis, and well as the anti-inflammatory and antithrombotic effect that influences their therapeutic application. Statins were found to reduce the risk of cardiovascular complications [5] and

* Corresponding author.
E-mail address: wojciech.slupski@umed.wroc.pl (W. Słupski).

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overall mortality regardless of the initial level of serum cholesterol [6]. Results of experimental and clinical studies of statins on central nervous system (CNS) disorders, including depression, anxiety, Alzheimer's and Parkinson's disease were inconclusive. Simvastatin is one of the most popular drugs belonging to that group. It was suggested that the drug may influence the CNS function lowering cholesterol synthesis in the system, and exerting some non-lipid-lowering (pleiotropic) mechanisms [7]. Experimental [8–11] and clinical studies [12] have suggested that simvastatin through its influence on the activity of the *P*-glycoprotein 1 (P-gp) and cytochrome P450 isoenzymes (especially CYP3A4) could modify the pharmacokinetics of other drugs, including diazepam.

There are differences in the metabolism of diazepam in humans and in rats. The main metabolite of diazepam in humans is *N*-desmethyldiazepam, the pathway of which is mainly catalyzed by CYP2C19 and to a lesser extent by CYP3A4/5 and CYP2B6. In smaller amounts, 3-hydroxydiazepam (temazepam) is produced by CYP3A/4, which besides *N*-desmethyldiazepam and 4-hydroxydiazepam (*p*-hydroxydiazepam), is the major metabolite in the Wistar rats. Temazepam formation is catalysed by CYP3A/2. CYP2C11 and CYP2D3 catalyze the conversion of diazepam to 4-hydroxydiazepam and *N*-desmethyldiazepam, respectively [13]. Considering the fact that a large group of patients treated with simvastatin has also an indication for the acute use of diazepam, the analysis of potential interactions between these two drugs seems highly important and may potentially increase the safety of that combined therapy. The aim of this study was to evaluate the effect of simvastatin administered chronically on the anxiolytic action of diazepam and its pharmacokinetic properties in rats.

Materials and methods

Animals

The experiment was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Local Ethics Committee. Male Wistar Han rats, approximately 10 weeks old, were housed in pairs (in every cage) with a 12:12 h light-dark cycle and a free access to standard food and water. The room temperature was maintained at 21–23 °C. Before and during the treatment period, body weight of animals was measured. Each experimental group consisted of 8–10 animals.

Drug administration

Phase 1

Simvastatin (Polpharma S.A., Starogard Gdański, Poland) was suspended in a 1% aqueous solution of Tween 80 (POCH, Gliwice, Poland). Diazepam (Relanium, Polfa, Warszawa, Poland) was diluted with 0.9% NaCl with one drop of Tween 80. Simvastatin was administered orally (po) for 4 weeks at the once daily dose of 2.5, 5, 10, 20 mg/kg (groups: S2.5, S5, S10, S20). Diazepam was administered *po* at the once daily dose of 2.5, 5, 10 mg/kg (groups: D2.5, D5, D10) on each day of behavioral research. Control group (C1) received *po* 1% aqueous solution of Tween 80 (*vehiculum).* Drugs and *vehiculum* were administered in a volume of 4 ml/kg.

Phase 2 (pharmacodynamic interaction study)

The doses of examined substances were selected on the base of results from the first phase of the experiment. Four combinations

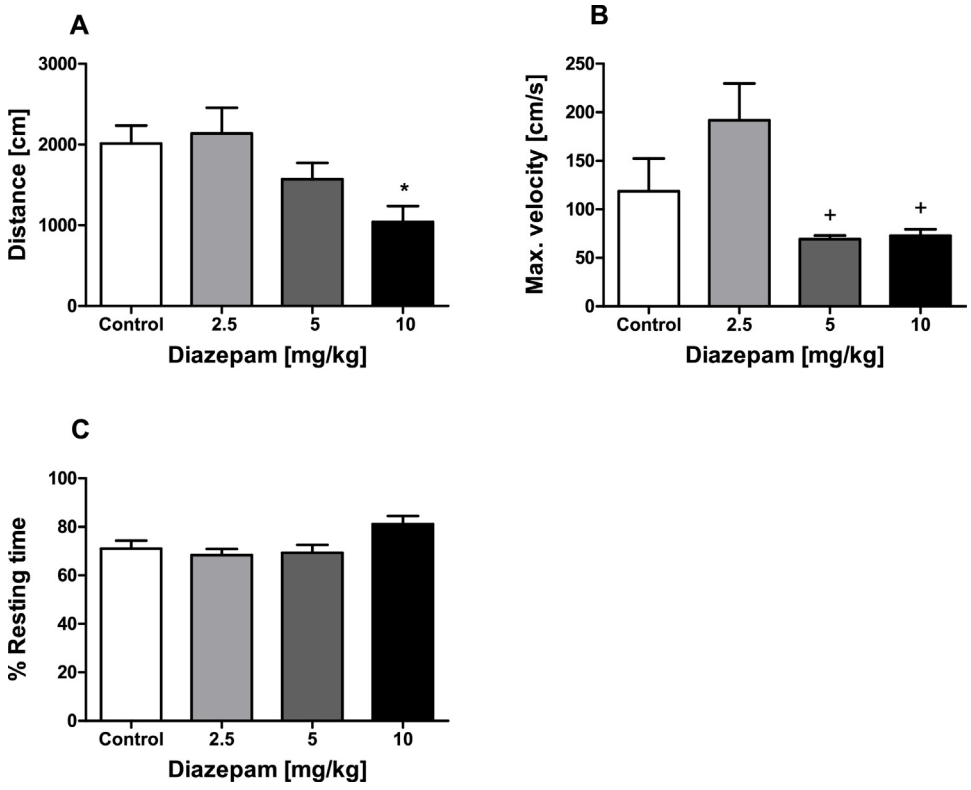

Fig 1. Effects of diazepam on the distance traveled (A), the maximum speed of movement (B) and the percentage of resting time (C) in the open field test. Histogram represents the mean (±SEM); * $p < 0.05$ significantly different from the control group (Dunnett's test), + $p < 0.05$ significantly different from the group of 2.5 mg/kg (Tukey test).