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Effect of Pioglitazone on Plasma Levels of Phenytoin in Rats

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ABSTRACT

Introduction: Interaction between drugs represents a major clinical concern for health care professionals and their patients. Patients affected by both type 2 diabetes and epilepsy may be prescribed pioglitazone and an anti-epileptic drug such as phenytoin concurrently. The aim of this study was to consider the interaction of pioglitazone with phenytoin in an experimental model. According to the result of this study concurrent use of phenytoin and pioglitazone in clinic may cause therapeutic failure of phenytoin which may cause seizures and during seizures the cardiac function may be affected.

Materials and Methods: Two groups of rats were treated for 30 days. In group 1 (control group) saline (10 ml/kg) and phenytoin (30 mg/kg) were administered daily by intragastric gavage. In group 2 (test group), pioglitazone (10 mg/kg) was administered daily 60 minutes before phenytoin (30 mg/kg). Two hours after the last intragastric gavage, animals were anesthetized with ether and 2 ml of blood was drawn from the heart into a syringe containing Ethylenediaminetetraacetic (EDTA), and phenytoin concentration in rat plasma was determined by High performance liquid chromatography (HPLC).The study consisted of 2 groups of 10 male adult Wistar rats.

Results: Compared with control group, concurrent use of pioglitazone and phenytoin was associated with significantly lower mean plasma concentrations of phenytoin: 2.08 ± 0.25 µg/ml *VS* 1.2 ± 0.02 µg/ml ($p< 0.05$).

Conclusion: Concurrent use of pioglitazone and phenytoin was associated with a significant decrease in plasma concentration of phenytoin in this experimental model. In clinic, this interaction may cause seizures and it has been shown that both cardiac and respiratory functions may affected by seizures.

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Introduction

Epilepsy is one of the most common serious neurological conditions. Both cardiac and respiratory functions are affected by seizures (1). Patients with epilepsy have a 2-5 fold suceptible in the occurrence of the conditions, including cardiovascular and pulmonary disorders. Among epilepsy patients, asthma is common between the young, while cardiovascular diseases and stroke are prevalent in older individuals, but both occur more frequently in the general population. Phenytoin (5,5-diphenylhydantoin) is a widely used antiepileptic drug with effecctive plasma

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level between 10 and 20 µg/ml. Pioglitazone is a antidiabetic and insulin-sensitizing agent of thiazolidinedione group with high-affinity to PPAR-γ (peroxisome proliferator-activated receptor). It is thought that Pioglitazon activity on PPAR is a primary machanism by which this drug exert its antidiabetic effect (2). Pioglitazone as a PPAR-γ agonist also has beneficial effect on the treatment of neurodegenerative disorders like Alzheimer and it has been proposed as a neuroprotective agent disease (3). It may be used concurrently with phenytoin in patients with epilepsy who are also affected by neurodegenerative disorders and diabetes melitus. Drug-drug interactions occur when one therapeutic agent either alters the concentrations or the biological effect of another agent. Due to ethical constraints, ralevant pharmacokinetic and metabolism studies must be carried out extensively in laboratory animals or in vitro systems before first drug adminstration in humans (4). Phenytoin is metabolized by Cytochrom P (CYP) 450 family of enzymes like CYP2C9, CYP2C19 and UDP-glucuronosyltransferases (UGTs) like UGT1A9 (5). Several studies have confirmed that PPAR activators affect the gene expression or/and enzyme activity of various CYP family of enzymes or conjugating enzyme and regulate hepatic and extrahepatic metabolism pathways (6, 7, 8). It has been shown that UDP-glucuronosytrans-ferase1A9 enzyme is a PPARs target gene (9) and thiazolidinediones such as pioglitazone act as PPAR-γ ligand (2). The aim of this study was to investigate the drug-drug interaction between pioglitazone and phenytoin in male wistar rats.

Materials and Methods

The following drugs and chemical were used in this study: Phenytoin sodium (Caspian Tamin Co., Rashat Iran), Pioglitazone (Osveh Co., Tehran, Iran), HPLC (High performance liquid chromatography) grade methanol, 2-propanol, acetonitrile and propylene glycol were purchased from Scharlau (Barcelona, Spain) and analytical grade sodium hydrogen mono-phosphate and EDTA (Ethylenediaminetetraacetic) were obtained from Merck (Darmstadt, Germany). Double distilled water was used for all preparations. All drugs were dissolved in propylene glycol: water mixture (50:50). Drugs were administrated orally through a tube gavage.

Male Wistar rats (270-300 g at the beginning of the experiment) were bred in the animal house of Drug Applied Research Center, Tabriz University Medical Sciences. The animals were kept in a temperature/humidity controlled environment and subjected to natural daily dark-light cycle with free access to rodent chow and tap water. All animal experiments were accomplished between 9 AM and 1 PM and the rats were excluded from the experiment whenever resisted swallowing the drugs. Experiments were performed in accordance with the guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985) and were approved by the Research and Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran.

The rats randomly divided into control and test groups. Each group consisted of ten animals. In control group, saline (10 ml/kg) and phenytoin (30 mg/kg) were administered daily by intragastric gavage (10, 11). In test group, pioglitazone (10 mg/kg) was administered daily 60 minutes befor phenytoin (30 mg/kg). Pioglitazone dose was chosen based on its established effective dose in nuroprotection (12). Animals were anesthetized with ether and 2 ml of blood was drawn from the heart into a syringe containing EDTA with a final concentration of 1g/L, and phenytoin concentration in rat plasma was determined by HPLC. Two hours after the last intragastric gavage, blood sample transferred to plastic tubes and centrifuged at 13000 rpm min^{-1} for 10 min and the plasma separated and stored at -70 °C until HPLC analysis to determine plasma concentrations of phenytoin. Plasma samples proteins were precipitated using acetonitrile in 1:1 ratio and then supernatant was separated using centrifuge at 7000 rpm for 7 min. The concentrations of phenytoin in rat plasma were determined using NovaPak® C_{18} analytical column (Milford, Ireland; 250 × 4.6 mm, 4 µm) and potassium hydrogen phosphate buffer (pH 6.0)/acetonitrile/2-propanol (63:22:15, v/v/v) as the mobile phase, at a flow rate of 1.0 ml/min and UV detector (13).

Values were reported as mean ± SE. Independent T-test was used to determine mean differences and $p<0.05$ was considered statistically significant. Calculations were performed using Statistical Package for Social Sciences (SPSS version11.5, Chicago, IL, USA).

Results

The study comprised 2 groups of 10 male adult Wistar rats each. All animals survived up to the end of study.

Animals in group 1 (control group) were treated with phenytoin (30 mg/kg/day) and plasma level of phenytoin was determined after 30 days by HPLC. As Table 1 shows, plasma level of phenytoin has been 2.08 ± 0.25 µg/ml.

Animals in group 2 (test group) pretreated with pioglitazone (10 mg/kg/day) 60 minutes before phenytoin administration and plasma level of phenytoin was determined by HPLC after 30 days.

Compare with control group, concurrent use of pioglitazone was accociated with significantly lower plasma phenytoin (1.20 ± 0.02 µg/ml, $p<$ 0.05, Table 1).

Table 1. Mean plasma levels of phenytoin after subchronic administration (30 mg/kg/day) in control and test groups of rats.

Subgroups (n=10)	Phenytoin Plasma level (µg/ml)
Control group	2.08 ± 0.25
Test group	1.20 ± 0.02*

*P<0.05 versus control group

Discussion

In clinical practice, it is not possible to prevent co-prescription of different drugs with clinical significant interactions and this is why the understanding of the drug interactions is so important (4). The CYP450 enzyme family plays a determinant role in the metabolism of a vast number of drugs. Many drug interactions are a result of inhibitions or induction of CYP enzymes. Our results show that sub-chronic conccurrent administration of pioglitazone with phenytoin was associated with significant reduction in plasma concentration of phenytoin in this experimental model (P<0.05) and this effect may be related to overexpression of the gene that code the UDP-glucuronosytransferase 1A9 (UGT 1A9). Optimum control of seizures without clinical signs of toxicity occurs more often with serum levels between 10-20 µg/ml of phenytoin. Patients with epilepsy may be predisposed to the acute epileptogenic disturbance of autonomic function and subsequent cardiac arrhythmias due to the effects of recurrent seizures on cardiac microstructure. In patients taking anti-epileptic medication, drug-drug interaction between pioglitazone and phenytoin may cause therapeutic failure of phenytoin which may cause recurrent seizures and sudden unexpected death epilepsy (SUDEP) (14).

In clinic this interaction may cause therapeutic failure of phenytoin and may need a better management of pharmacotherapy.

Large and controlled studies in humans are needed to confirm these results.

Conclusion

Concurrent use of pioglitazone and phenytoin was associated with a significant decrease in plasma concentration of phenytoin in this experimental model. This interaction may be related to overexpression of the target gene.

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Conflict of Interest

The authors have no conflict of interests.

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