Insulin potentiates the anticonvulsive activity of phenytoin against maximal electroshock-induced seizures in mice

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Abstract

Background and Aim: The limitations imposed by the blood–brain barrier (BBB) on the sufficient accumulation of antiepileptic drugs (AEDs) in the epileptogenic focus is considered the major cause of the high percentage of morbidity and mortality cases among epilepsy patients. This study aimed to examine the potential effect of insulin on the anticonvulsant action of phenytoin (PHT) in the mouse maximal electroshock-induced seizure model.

Materials and Methods: PHT was administered orally in single doses either alone or in combination with insulin given as single intraperitoneal injections. To assess the anticonvulsant activity of PHT, the ED50 values were calculated. The current strength (CS_{50}) threshold for insulin was also estimated. The animals were sacrificed, and the brains were removed to measure their PHT concentrations in the brain.

Results: It has been demonstrated that insulin (in all used doses) has no effect on the $CS_{50,}$ but can cause a significant increase in concentrations of PHT in the brain and potentiate the antiepileptic efficiency of this drug in electroshock-induced models of epilepsy in mice.

Conclusion: The combination of insulin with PHT may be of great importance for developing new treatment possibilities following further investigations with other animal models of epilepsy and preclinical studies. Further research is also needed to explore the concentrations of PHT in the brain and the anticonvulsant activity of this drug against maximal electroshock seizures in diabetic mice.

Keywords:

Antiepileptic Drug, Epilepsy, Insulin, Phenytoin.

Introduction

The fundamental problem in the action of AEDs including PHT is their limited penetration through the BBB[1]. Approximately one-third of patients with epilepsy is estimated to develop resistance to the AEDs and have refractory epilepsy[2], and this is mainly attributed to the BBB, which is an obstacle for these drugs and inhibits their therapeutic effects[3]. Consequently, increasing the transport of AEDs to the brain represents a potential method for managing refractory epilepsy[4]. Moreover, most of AEDs including the sodium channel blocker PHT[5] can simultaneously accumulate in brain tissue and significantly distribute to other organs such as the liver, kidney, lung, and bone marrow. PHT can frequently induce significant adverse effects on these organs, which worsens refractory epilepsy and increases the decline in these patients' condition; this continues to be a critical clinical problem[6]. Thus, the clinical use of PHT has frequently been restricted due to chronic toxicity, which includes leukopenia, megaloblastic anaemia, aplastic anaemia, liver necrosis, rash, and hepatotoxicity[7]. Furthermore, the Stevens Johnson syndrome and toxic epidermal necrolysis were found to be linked with phenytoin[7],[8].

Improving the approaches to increase drug entry into the brain across the BBB is a crucial research area[9]. Current studies primarily target the passage process, by either adding a drug carrier or modification of the drug passage mechanism[10].

Thus, it is thought that the efficacy of PHT can be increased by the selective accumulation of this drug in the brain. This can be attained by increasing its penetration through the BBB and/or the blood–cerebrospinal fluid barrier, by enhancing the permeability of these barriers. Previous research showed that the transport of some drugs through different biological barriers (especially the BBB) can be increased using some peptides, particularly peptide hormones such as insulin[11]. This peptide shows specific tissue affinity, which means that it causes transport activation of a particular drug only to some tissues or organs, decreasing its concentration in others[11]. To date, PHT has not been investigated in this aspect, although the increase in its transport and action may have an enormous significance—both theoretically and clinically.

Therefore, the aim of this study was to investigate the influence of insulin on the action of PHT and to determine whether the administration of insulin will increase the action of PHT and its penetration through the BBB. PHT is a basic and widely used anti-epileptic/anti-seizure medication, and it was chosen because of its known limited accumulation in the brain[12].

Materials and methods

Animals

The experiments were performed on male outbred Ipf-Miz mice weighing 20–25 g. After 1 week of acclimatization to laboratory conditions, experimental groups consisting of eight to ten animals each were randomly selected. The mice were kept in conditions that were compliant with Good Laboratory Practices requirements (Such as 15-fold air change, fully automatic atmospheric air-conditioning, light change every 12h:12h light: dark cycle, and standard Murigran feed and water *ad libitum*). All experiments were conducted between 08:00 and 13:00 h. The tests were performed under standardized housing conditions (Temperature, 20 ± 1°C; relative humidity, 55 ± 5%).

The protocol of this study was approved by the ethics board of animal experiments, University of Hail, Saudi Arabia. All experimental procedures were conducted according to the guidelines set by the World Health Organization (Geneva, Switzerland).

Drugs

PHT and insulin were used (both from POLFA, Warsaw, Poland). PHT was suspended in 0.5% carboxymethylcellulose solution and administered orally at a dose of 0.2 ml/10 g body weight (b.w.). Insulin was dissolved in 0.9% NaCl and administered intraperitoneally (i.p.) at a dose of 0.5, 1, or 2 I.U./kg b.w. The 40% glucose solution was administered orally at the same time as insulin at a dose that ensured normoglycaemia. In the control group, sterile saline (instead of insulin) and distilled water (instead of glucose) were administered. New drug solutions were made each testing day and administered as follows: PHT at 2.0 hours and insulin and glucose solution at 1 hour (our unpublished results

demonstrated that this period is essential for insulin to exert its maximum effect on PHT activity) before electroconvulsions and brain sampling to measure PHT concentrations.

Electroconvulsions

Electric shocks were induced using an alternating current stimulator (Rodent Shocker, Hugo Sachs Elektronik, Freiburg, Germany), which provided a frequency of 50 Hz and a stimulus duration of 0.2 s, using ear-clip electrodes. This stimulator has internal stabilization, which means that each mouse received the same current, regardless of the resistance. The evaluation criterion was direct tonic convulsion in the hindlimbs. Initially, the current strength (CS_{50}) threshold was determined, i.e., the current intensity (in mA) that induces a tonic convulsion directly in the hindlimbs in 50% of mice. The ED_{50} value (in mg/kg), i.e., the dose of a given drug that protects 50% of animals against tonic convulsions induced by the maximum electroshock seizure (MES), was determined. The MES current for the apparatus used was 25 mA (approximately five times the convulsive threshold for mice). At least four groups of animals (n=10 mice/group) were used to determine ED_{50} or CS_{50} values.

Blood glucose testing for animals

This test was aimed to determine the glucose dose that was needed to normalize hypoglycaemia induced by administering 0.5, 1, or 2 I.U./kg b.w. The tests were performed using the DIASCAN-S apparatus following the manufacturer's instructions. Normalization of hypoglycaemia with the administration of 0.5 I.U./kg b.w. required 0.35 ml of 40% glucose/mouse, 1 I.U./kg b.w. required 0.6 ml of 40% glucose/mouse, and for 2 I.U./kg b.w. required 1 ml of 40% glucose/mouse.

Measurement of the total brains PHT concentrations

The total PHT concentration was determined in the brain of animals receiving PHT, insulin, and a 40% glucose solution. The total brain concentrations of animals receiving PHT, its solvent (0.9% NaCl solution), and distilled water was also determined. Mice were sacrificed by decapitation at times based on the MES test. The brains were removed, weighed, and homogenized using an Abbott buffer (2:1 v/w) in Ultra-Turrax T8 homogenizer (Staufen, Germany). The homogenates were centrifuged at 10,000 ×*g* for 10 min, and 75 ml of supernatant were placed into the Abbott system cartridges. The total brain's PHT

concentrations were analysed by fluorescence polarisation immunoassay using a TDx analyser and reagents, according to the manufacturer's instructions (Abbott Laboratories, Chicago, IL, USA). Total brain concentrations were measured in μg/ml for the brain supernatants and presented as the mean ± standard deviation (SD).

Statistical analysis

The probit analysis[13] was used to determine the ED50 value (in mg/kg), CS50 (in mA), confidence intervals (presented in the tables), and statistical significance. Total brain concentrations of PHT administered alone or in combination with insulin were statistically analysed using the Student's *t*-test, and the arithmetic means and SD (presented in the tables) were determined in each group. $p < 0.05$ was considered statistically significant.

Results

Effect of insulin on the convulsive threshold

Insulin (administered alone, i.p., 60 min before the test) at doses of 0.5, 1, or 2 I.U./kg b.w. did not affect the seizure threshold in the MES test in mice. These results are shown in Table I.

Effect of insulin on the anticonvulsant activity of PHT

Table II displays the ED50 values for PHT. Co-administration of PHT with either 0.5 I.U./kg or 1 I.U./kg of insulin did not significantly enhance the anticonvulsant activity of the former drug (although it decreased its ED50 value from 10.4 to 8.7 and 8.0 mg/kg, respectively). However, insulin at a dose of 2 I.U./kg significantly ($p<0.01$) potentiated the anticonvulsant activity of PHT against the MES test reducing its ED50 to 6.1 mg/kg (Table II).

Effect of insulin on the total brain concentration of PHT

The total brain concentration of PHT (6.1 mg/kg) administered alone did not significantly differ from that determined for the combination of PHT (6.1 mg/kg) and 0.5 or 1 IU/kg insulin. However, insulin at a dose of 2 I.U./kg significantly raised the brain concentration of PHT ($p< 0.05$; Table III). In this case, insulin increased the total brain PHT concentrations from 0.88 to 1.21 μg/ml.

Discussion

The findings of this study indicate that insulin significantly potentiated the anticonvulsant action of PHT against MES-induced seizures in mice in a dose-dependent manner. These results are consistent with previously reported results, which indicated that insulin increases both the pharmacological activity and tissue accumulation of several drugs (e.g., chlorpromazine) in the central nervous system (CNS), which is thought to be due to an increase in BBB permeability[11]. However, the current results contradict previous results, which showed that insulin decreases the anticonvulsant action of carbamazepine during the MES test in mice and the accumulation of this agent in the brain by decreasing its penetration through the BBB[14].

Insulin used in this and the other above-mentioned studies was administered with a sufficient amount of glucose to ensure normoglycaemia. Similarly, the insulin doses used in this research did not affect the convulsive threshold in mice. These findings are consistent with those of other studies[14]. However, insulin markedly increased the total brain PHT concentration, which may have occurred by enhancing the permeability of BBB for this drug.

Moreover, it was previously demonstrated that if insulin (during normoglycaemia) increased the activity of some drugs and their brain concentrations, the action and concentration of these drugs would significantly decrease in experimental diabetic animals[11]. Based on these findings and the results of the current study, it is reasonable to speculate that the anticonvulsant action and the brain concentration of PHT could be significantly reduced in diabetic individuals. The results of a clinical study revealed that PHT blood concentrations were significantly lower in diabetic patients compared to the controls[15].

To determine the cause of this contrasting effect of insulin on carbamazepine compared to its effect on PHT, the molecular mechanisms that support the effects of insulin on BBB permeability and the mechanism of PHT penetration through BBB compared to that of carbamazepine should be considered. Insulin may have different effects on the membrane transport proteins at the BBB, meaning that it may stimulate or inhibit uptake transporter and/or enhance or constrain efflux transporters. It is also possible

that PHT penetration through the BBB and into the brain occurs through a different BBB transporter system than carbamazepine.

Insulin has been shown to modify cell proliferation and tight-junction integrity in hCMEC/D3 cells at the BBB and enhance the action of ATP-binding cassette efflux transporters in these cells, leading to an increase in beta-amyloid clearance[16, 17]. In this manner, insulin may be involved in preserving the BBB function[18].

These efflux transporters play a crucial role in the central distribution of many AEDs, including carbamazepine[19], and thus, insulin may decrease the activity and brain concentration of carbamazepine by enhancing the activity of these efflux transporters[14].

PHT was believed to be a substrate of ATP-binding cassette transporters, specifically P-glycoprotein[20]. However, it has been recently revealed that monocarboxylate transporter 8 (MCT8), rather than P-glycoprotein, is responsible for PHT efflux transport across the BBB[21].

No data were found about the potential effects of insulin on the MCT8 transporter. However, insulin-like growth factor-1 was shown to significantly affect the function of MCT8[22]. Thus, it can be speculated that insulin increases the brain concentration of PTH by inhibiting the MCT8 transporter.

The pharmacokinetic estimation of total PHT in the brain in the current study is important because it helps to determine the nature of the detected interactions between drugs in the MES test. Additionally, only the total AED brain concentration can accurately illustrate the pharmacokinetic interactions between drugs influencing the CNS[23]. Thus, in the present study, the total brain concentration of PHT was evaluated, rather than its free plasma concentration.

Conclusion

In conclusion, insulin potentiated the anticonvulsant action of PHT and increased the total brain concentration of this drug in experimental animals. This, in turn, may lead to new treatment opportunities after further experimental and preclinical studies. Additionally, both the anticonvulsant action and the brain concentration of PHT warrant further investigation in experimentally induced diabetic animals.

Conflict of Interest

The Authors declare that they have no conflict of interests.

ACKNOWLEDGEMENTS:

The authors are thankful to the Middle East University, Amman, Jordan, for the financial support granted to cover the publication fee of this research article.

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Table I. Effect of insulin on the electroconvulsive threshold

Treatment (I.U./kg)	CS_{50} (mA)
Vehicle	5.8 (5.6–5.9)
Insulin (0.5)	5.6 (5.4–5.8)
Insulin (1)	5.62 (5.5–5.9)
Insulin (2)	5.82 (5.7–5.9)

CS_{50} (in mA) is the current strength that produces convulsions in 50% of animals tested. Insulin was administered intraperitoneally 60 min before the electroconvulsions.

Table II. Effects of insulin on the anticonvulsant activity of phenytoin

Treatment (IU/kg) + (ml/mice)	ED_{50} (mg/kg)
PHT + 0.9% NaCl + distilled water	10.4 (8.7–12.4)
PHT + insulin (0.5) + 40% glucose solution (0.35)	8.7 (6.8–10.2)
PHT + insulin (1) + 40% glucose solution (0.6)	8.0 (6.0–9.5)
PHT + insulin (2) + 40% glucose solution (1)	6.1 (4.0–7.5)**

Results are presented as the ED_{50} (median effective doses) values (in mg/kg) with 95% confidence intervals in parentheses. ED_{50} values and statistical comparisons were calculated according to Litchfied and Wilcoxon [12]. PHT was administered orally 120 min before the MES-induced seizures.

PHT, phenytoin; ED_{50}, the dose of a medication that produces a specific effect in 50% of the population that takes that dose. $^{**}p<0.001$ vs. the ED_{50} value of respective control.

Table III. Effect of insulin on total the phenytoin brain concentration

Treatment (mg/kg) + (I.U./kg)	Brain concentrations (μg/ml)
PHT (6.1) + 0.9% NaCl	0.88 ± 0.102
PHT (6.1) + insulin (0.5)	0.91 ± 0.108
PHT (6.1) + insulin (1)	0.94 ± 0.111
PHT (6.1) + insulin (2)	1.21 ± 0.112*

Results are presented as the mean ± SD. Data were statistically analysed using the unpaired Student's *t*-test. PHT was administered orally at a dose of 6.1 g/kg (the ED_{50} value for PHT when given with 2 I.U./kg insulin). The increase in total brain PHT concentration was compared with the control group.

*P<0.05 vs. PHT+ vehicle-treated animals.

PHT, phenytoin; SD, standard deviation; ED_{50}, the dose of a medication that produces a specific effect in 50% of the population that takes that dose