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Original Research

Glucose Lowering Effect of Parenteral Doses of Phenformin of Expected Low Serum Perturbations

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Phenformin is a biguanide antidiabetic agent that is no longer commonly in use due to lactic acidosis. In this study, low doses of phenformin 50 mg/kg were administered intraperitoneally to rats and the blood serum glucose levels were monitored and compared with those for oral doses (200 mg/kg). The serum of the rats was also monitored for changes in pH. The results indicate that the 50 mg/kg of phenformin lowered blood glucose levels to the values achieved with the oral dose. The pH of the serum also remained constant and did not lower throughout the duration of the experiments. Metformin was also administered orally for the purpose of comparison. It is believed from this study that low parenteral doses of phenformin might be an alternative to the large oral doses.

Key words: Glucose lowering effect, parenteral administration, phenformin, low serum perturbations.

Introduction

The biguanides are a group of compounds that are commonly used in the treatment of type 2 non-insulin dependent diabetes mellitus. Two major members of the group are used for this purpose-metformin and phenformin [1]. Phenformin, however, no longer enjoys wide therapeutic use due to a number of deaths as a result of lactic acidosis [2]. The drug however is still used in clinical practice in limited cases where adequate medication may not be available [3, 4].

Large doses of certain drugs are often used when the oral route is the choice for drug administration. This is because of the unpredictable bioavailability associated with drug administration via this route. This unpredictable bioavailability has been reported for the biguanides [5]. They are known to adhere to the stomach wall after oral doses necessitating the use of large doses to enable sufficient therapeutic concentration to be maintained in the blood [6].

Toxicity of a drug is often related to the dose. Low doses of a drug are less likely to produce toxic effects than hgih doses. However, such low doses may not achieve the desired therapeutic response unless a suitable route of administration is selected. In this study, low doses of phenformin are administered via the intraperitoneal (IP) route to rats and blood glucose lowering effects determined. The results are compared with high doses of metformin and phenformin. IP route of drug delivery has been used for over 20 years to target potent chemotherapeutic agents in the treatment of certain types of cancers [7]. In addition, IP administration has been shown to reduce the hematologic toxicity of carboplatin compared with intravenous administration [8].

Materials and Methods

Materials

The following materials were used as procured from their manufacturers without further purification: phenformin, metformin and alloxan (Sigma, USA), One Touch Glucometer (Accuchek, UK) and prosopis gum prepared in our laboratory. Distilled water was obtained from a glass still.

Animals

Male Wistar rats (weighing between 260-280 g) bred in the animal unit of the Department of Pharmacology and Toxicology were used for the

tests. All the rats were fed with standard laboratory diets. All experiments were carried out according to the "International Guidelines on the Use of Laboratory Animals for Biomedical Research".

Methods

Induction of diabetes

Experimental diabetes was induced in the rats using alloxan 150 mg/kg body weight. Five days after administration, blood samples were withdrawn from the jugular vein of the rats after fasting for 18 h and examined for glucose content using a glucometer. Fasting blood glucose levels of at least 200 mg/dl were taken as the diabetic base line.

Antidiabetic studies

A solution of the drug was made in sterile normal saline. A dose equivalent to 50 mg /kg body weight was administered IPly to four diabetic rats which have been fasted for 18 h. The rats were anaesthetized with a solution of phenobarbitone sodium (50mg/ml, determined experimentally). Blood samples were then withdrawn hourly from the jugular vein of the rats and analysed for glucose content using a glucometer. The pH of the blood samples were also checked using a pH meter (model M-11, Horiba) using calomel electrodes (model 6069-10C, Horiba). For a delayed preparation, a similar dose was dispersed in prosopis gum dispersion (1% w/v) in sterile normal saline and administered to another four set of rats and treated similarly as the first four. The next group of four rats was given oral doses of phenformin (200 mg/kg) while another set received 200 mg/kg of metformin. The last set of four rats was given only normal saline (1 ml). All the tests carried out on the blood samples from first rats were carried out on all the others. The areas under the effect versus concentration time curves (AUEC) of the materials were computed using trapezoidal rule and based on the non-compartmental approach [9]. Analysis of variance was used to statistically test the results in terms of differences in the results.

Results and Discussion

Table 1 shows the results of the pH measurements on the blood samples treated with the drug samples. The pH did not alter throughout the test period. This is indicative of lack of serum perturbations. Direct measurement of the pH of blood samples may be used to access lactic acidosis. When this approach is used, the pH of the blood should not fall below 7.4 [10]. However, it may be wrong to conclude based entirely on this result because a more chronic administration for weeks or even months may change the picture of the level of acidaemia.

Table 1. Mean pH values of rat blood during the experiments

Material	Time (h)					
	0	2	4	6	8	10
Normal saline (1 ml)	7.70(0.03)	7.85(0.02)	7.75(0.03)	7.80(0.01)	7.90(0.01)	7.75(0.02)
I.P. phenformin (50 mg/kg)	7.70(0.03)	7.75(0.03)	7.75(0.03)	7.80(0.03)	7.80(0.03)	7.75(0.02)
Oral phenformin (200 mg/kg)	7.70(0.03)	7.85(0.01)	7.70(0.02)	7.75(0.02)	7.85(0.02)	7.85(0.02)
Oral metformin (200 mg/kg)	7.70(0.03)	7.80(0.02)	7.85(0.01)	7.75(0.02)	7.80(0.01)	7.80(0.01)
I.P phenformin in Prosopis dispersion (50mg/kg)	7.70(0.03)	7.70(0.03)	7.70(0.02)	7.70(0.02)	7.70(0.01)	7.70(0.01)

n=4; SD values are in parenthesis

Fig. 1 shows the plasma glucose levels of the rats during treatment with the drugs. The plasma glucose levels decreased progressively after administration and returning back to normal after the third hour in some groups of the aminals. The lowering of blood glucose levels caused by the oral doses of the two antidiabetic drugs was not significantly different ($p<0.5$) to those of the IP injections. A clearer picture is produced by the values of other AUECs shown in Table 2.

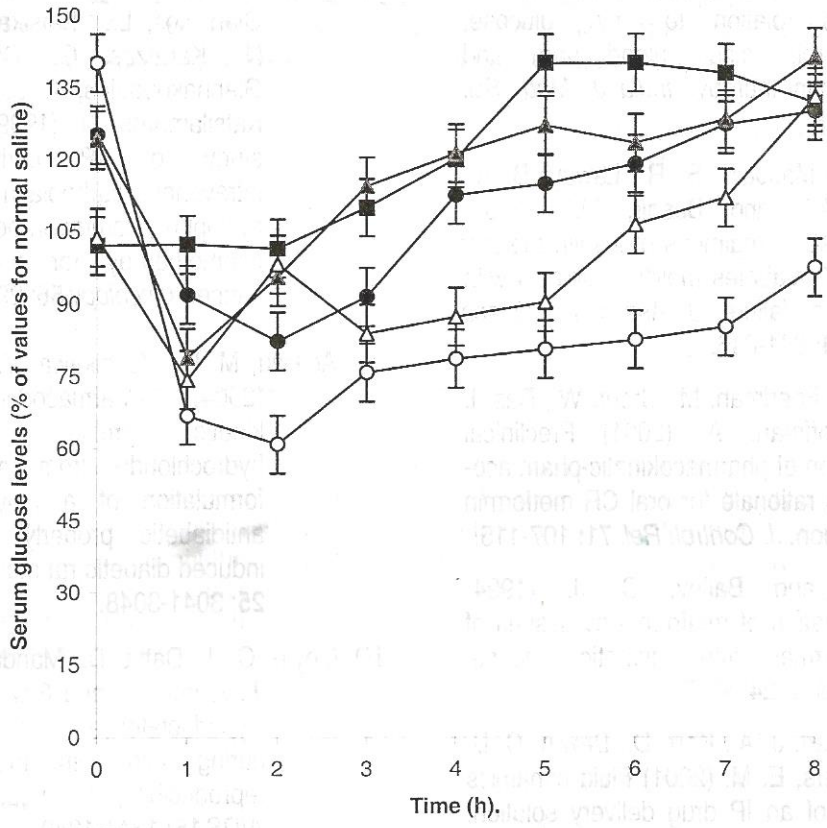

Fig. 1: Effect of the various preparations and modes of administration on serum glucose levels of diabetic rats. o phenformin (i.p 50 mg/kg); Δ phenformin (p.o. 200 mg/g); • metformin (p.o. 200 mg/kg); ▲ phenformin/prosopis gum (i.p. 50 mg/kg), ■ normal saline.

Table 2. Pharmacodynamic properties of the preparations.

Preparation	AUEC (% h)	Nadir effect (%)
Normal saline (1 ml)	Nil	Nil
I.P. phenformin (50 mg/kg)	716.47 ± 8.91*	39.12 ± 7.38
Oral phenformin (200 mg/kg)	697.36 ± 17.44	18.00 ± 6.62
Oral metformin (200 mg/kg)	627.02 ± 6.81	22.05 ± 6.00
I.P phenformin in Prosopis dispersion (50mg/kg)	541.02 ± 28.27	17.07 ± 9.18

*n=4; *Significantly different from the oral controls at $p < 0.05$*

From the AUEC values, the IP phenformin has advantage over the oral route, as this value is indicative of the total effectiveness of the drug. Similarly, the nadir effect (which is the percentage depression in glucose level calculated as percent using normal saline as the baseline) also indicated that the peritoneal injection of phenformin produced the highest depression in blood glucose level. The values obtained with the dispersion of prosopis gum were lowest. The dispersion was intended to produce a delayed absorption of the drugs from the site of injection.

It is clear from these results that the parenteral route should be considered as alternative route for the administration of phenformin.

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