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Brief Communication

Influence of atazanavir on the pharmacodynamics and pharmacokinetics of gliclazide in animal models

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ABSTRACT

Background: The objective of this study was to investigate the effect of atazanavir on the pharmacodynamics and pharmacokinetics of gliclazide in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination.
Methods: Blood samples were analysed for blood glucose by GOD/POD method, serum gliclazide levels by HPLC method and insulin by Radio Immuno Assay method.
Results: In combination, atazanavir significantly enhanced the pharmacodynamic activity and altered the pharmacokinetic parameters of gliclazide in animal models.
Conclusions: The interaction between atazanavir and gliclazide appears to be pharmacokinetic interaction at metabolic level in animal models.

1. Introduction

The study of mechanisms of drug interaction is of much value in selecting drug concentrations to provide rational therapy. Drug interaction studies assume much importance, especially for drugs that have a narrow margin of safety, and where the drugs are used for a prolonged period of time. Diabetes mellitus is one such metabolic disorder that needs treatment for prolonged periods, and maintenance of normal blood glucose level is very important in this condition, since both hyperglycemia, as well as hypoglycemia, is unwanted phenomenon [1].

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism, and an increased risk of complications from vascular disease. Diabetes may be due to a decrease in the synthesis of insulin (type-1) or a decrease in the secretion of insulin (type-2) from the β-cells of islets of Langerhans of the pancreas. There are an estimated 143 million people world wide sufferings from diabetes [2] and the number may well double by the year 2030 [3]. In India, the prevalence rate of diabetes is estimated to be 1–5%.

Insulin resistance, impaired glucose tolerance and type-2 diabetes are conditions that are increasingly described in HIV-1 infected subjects receiving highly active antiretroviral therapy (HAART), especially with protease inhibitors (PIs) [4,5]. Atazanavir is a commonly prescribed protease inhibitor, due to its once-daily dosing regimen, favorable metabolic profile and low frequency of adverse effects [6]. However, its effect on oral antidiabetic therapy is not known.

Oral hypoglycemic agents are used in the treatment of type-2 diabetes, among which gliclazide, a second generation sulphonylurea derivative, is preferred in therapy because of its selective inhibitory activity towards pancreatic K^+ ATP channels [7], antioxidant property [8], low incidence of producing severe hypoglycemia [9] and other haemobiological effects. Gliclazide is known to act mainly by releasing insulin by blocking K^+ channels in the pancreatic β-cells [10].

Atazanavir is a substrate and potent inhibitor of the cytochrome P450 (CYP) system, in particular CYP3A4 and CYP2C9 and affect the metabolism of several drugs [11]. Because atazanavir can inhibit CYP3A4 and CYP2C9-mediated drug metabolism and gliclazide is reported to be metabolized by CYP2C9 primarily and partly by CYP3A4 [10,12], it is important to study the possible effects of atazanavir on the pharmacokinetics and pharmacodynamics of gliclazide. However, there seem to be no published studies on the effects of enzyme inhibition on the pharmacokinetics of gliclazide.

Since there is every possibility for the combined use of gliclazide and atazanavir in chronic diabetics with associated HIV infection, the study is planned to investigate the effect of atazanavir on the activity of gliclazide in normal and diabetic rats, to evaluate the

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safety and effectiveness of the combination. Also the study is planned to find the pharmacodynamics and pharmacokinetics of gliclazide in the presence of atazanavir in rabbits, to evaluate the mechanisms of interaction if they occur.

2. Material and methods

2.1. Drugs and chemicals

Gliclazide and atazanavir are gift samples from Micro Labs (Bangalore, India) and Aurobindo Pharma Ltd. (Hyderabad, India), respectively. Alloxan monohydrate was purchased from LOBA Chemie (Mumbai, India). Glucose kits (span diagnostics) were purchased from a local pharmacy. Acetonitrile (HPLC grade) was obtained from Qualigens chemicals, Mumbai, India. Orthophosphoric acid (AR grade) and dichloromethane (AR grade) were purchased from SD Fine Chemicals, Mumbai, India and Loba Chemie Pvt. Ltd., Mumbai, India, respectively. All other reagents used were of an analytical grade.

2.2. Animals

Albino rats of either sex, 6–7 weeks of age, weighing between 250 to 320 g, and normal albino rabbits of either sex of 3 months of age, weighing between 1.35 to 1.75 Kg, were used in the study. They were procured from the National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2 °C and 50 ± 15% relative humidity, with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet (Rayan's Biotechnologies Pvt. Ltd., Hyderabad, India) and water *ad libitum*. They were fasted for 18 h prior to the experiment, and during the experiment, the food and water were withdrawn. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee and by the Government regulatory body for animal research. (Reg. No. 516/01/A/CPCSEA). The study was conducted in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3. Selection of doses and preparation of oral test solution/suspension

In clinical practice, atazanavir and gliclazide in a therapeutic dose will be administered orally as antiretroviral and antidiabetic therapy, respectively. Human oral therapeutic doses of the respective drugs were extrapolated to rat/rabbit based on body surface area [13]. But the dose of gliclazide for rat experiments was selected as 2 mg/kg bd. wt. based on the influence of dose effect-relationship of gliclazide on blood glucose in normal rats. Atazanavir was suspended in 2% CMC-Na for oral administration [14]. Gliclazide solution was prepared by dissolving it in a few drops of 0.1 N NaOH then made up to the volume with distilled water. All the drugs were administered to the respective groups by oral gavage.

2.4. Pharmacodynamic interaction study in normal and diabetic rats

A group of six normal rats was administered with 2 mg/kg bd. wt. of gliclazide, orally. The same group was administered with atazanavir 36 mg/kg bd. wt., orally and the combination of atazanavir and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study, the same group was continued with the daily treatment of interacting drug (atazanavir) for the next 8 days with regular feeding. Later after 18 h fasting, they were again given the combined treatment on the 9th day.

The same treatment (single dose followed by multiple dose interaction study) was repeated in a group of six alloxan-induced diabetic rats. Diabetes was induced in rats by the administration of alloxan monohydrate in two doses, i.e. 100 mg and 50 mg/kg bd. wt. intraperitoneally for two consecutive days [15]. After 72 h, samples were collected from rats by orbital puncture of all surviving rats, and the serum was analysed for glucose levels. Rats with blood glucose levels of 200 mg/dl and above were considered as diabetic and selected for the study.

Blood samples were withdrawn from retro orbital plexus [16] of each rat at 0, 1, 2, 3, 4, 6, 8 and 12 h. These blood samples were analysed for blood glucose by GOD/POD method [17] using commercial glucose kits.

2.5. Pharmacodynamic and pharmacokinetic interaction study in rabbits

A group of six rabbits was administered with 5.6 mg/1.5 kg bd. wt. of gliclazide, orally. The same group was administered with atazanavir 28 mg/1.5 kg bd. wt., orally and the combination of atazanavir and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study the same group was continued with the daily treatment of interacting drug (atazanavir) for the next 8 days with regular feeding. Later after 18 h fasting they were again given the combined treatment on the 9th day.

Blood samples were withdrawn from the marginal ear vein of each rabbit at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h. These blood samples were analysed for blood glucose by GOD/POD method using commercial glucose kits. Plasma insulin was measured by Radio Immuno Assay method using a commercially available kit (human insulin as standard; Insik-5, Sorin Biomedica, Saluggia, Italy) as per the instructions provided by the manufacturers at 3 and 24 h. The serum gliclazide concentrations were determined by HPLC method [18]. The pharmacokinetic parameters of gliclazide were determined on subjecting the concentration-time data to non-compartmental analysis using WinNonlin (version 5.0.1) software.

2.6. Data and statistical analysis

Data were expressed as mean ± SEM. The significance was determined by applying Student's paired '*t*' test.

3. Results

3.1. Pharmacodynamic interaction study in normal and diabetic rats

Gliclazide produced hypoglycemic activity with maximum biphasic reduction of 40.88 ± 0.57% and 39.01 ± 0.73% in normal rats, and 42.95 ± 1.74% and 44.14 ± 1.78% in diabetic rats at 2 h and 8 h, respectively. Atazanavir has no significant effect on the blood glucose levels in normal and diabetic rats. In combination, atazanavir produced enhanced hypoglycemic effect of gliclazide with maximum blood glucose reduction of 48.27 ± 1.04% & 45.69 ± 1.53% and 50.14 ± 0.87% & 47.54 ± 1.00% at 2 h and 8 h, following single dose and multiple dose administration of atazanavir, respectively, in normal rats (Table 1). In combination, atazanavir produced enhanced hypoglycemic effect of gliclazide with maximum blood glucose reduction of 44.99 ± 1.13% & 48.00 ± 1.26% and 48.89 ± 1.18% & 50.25 ± 0.87% at 2 h and 8 h, following single dose and multiple dose administration of atazanavir, respectively, in diabetic rats (Ta-