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In vivo Effects of Gliclazide and Metformin on the Plasma Concentration of Caffeine in Healthy Rats

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Abstract: The *in vivo* effects of gliclazide and metformin HCl on plasma concentration of caffeine have been studied in rats. The plasma concentration of caffeine was determined by UV spectrophotometry after oral single administration of caffeine alone and with gliclazide and metformin HCl. The *in vivo* study for determination of plasma concentration of caffeine showed that concurrent administration of caffeine and gliclazide have not made noticeable changes in plasma concentration of caffeine. But administration of caffeine and metformin HCl has showed a significant change in plasma concentration of caffeine. So, a competitive inhibition of the binding to plasma protein by metformin HCI increases the plasma concentration of caffeine. Thus any change in plasma concentration may affect the pharmacological or toxic effects of the drug.

Key words: Caffeine, gliclazide, metformin, plasma concentration, rats

INTRODUCTION

Caffeine is a methyl xanthine that inhibits the enzyme phosphodiesterase and has an antagonistic effect at central adenosine receptors. It is a stimulant of the CNS, particularly the higher center and it can produce a condition of wakefulness and increased mental activity. It may also stimulate the respiratory center, increasing the rate and depth of respiration. Caffeine facilitates the performance of muscular work and increases the total work which can be performed by a muscle. Caffeine is used as a mild CNS stimulant in usual doses of 50 to 100 mg by mouth, although doses of up to 200 mg may be used. It is also frequently included in oral analgesic preparations with aspirin, paracetamol, or codeine in unit doses of 15 to 65 mg but its clinical benefit is debated. It is sometimes given with ergotamine for the treatment of migraine, usually in unit doses of 100 mg (Sawynok, 1995). Caffeine is absorbed readily after oral administration and is widely distributed throughout the body. It is also absorbed through the skin. Caffeine crosses the placenta. In adults, caffeine is metabolized almost completely in the liver via., oxidation, demethylation and acetylation and is excreted in the urine and other metabolites with only about 1% unchanged. Neonates have a greatly reduced capacity to metabolize caffeine and it is largely excreted unchanged in the urine until hepatic metabolism becomes significantly developed, usually by about 6 months of age. Elimination half-lives are about 3 to 7 h in adults but may be in excess of 100 h in neonates (Sweetman, 2005).

Gliclazide is a sulfonylurea antidiabetic molecule. It is given by mouth in the treatment of type 2 diabetes mellitus and has duration of action of 12 to 14 h. The usual initial dose is 40 to 80 mg daily and then gradually increased, if necessary, up to 320 mg daily (Sweetman, 2005). Gliclazide is readily absorbed from the gastrointestinal tract. It is extensively bound to plasma proteins. The half-life is about 10 to 12 h. Gliclazide is extensively metabolized in the liver to metabolites that have no significant hypoglycemic activity. Metabolites and a small amount of unchanged drug are excreted in the urine (Kobayashi *et al*., 1984).

Metformin hydrochloride is a biguanide antidiabetic molecule. It is given by mouth in the treatment of type 2 diabetes mellitus and is the drug of choice in obese patients. Initial dose is 500 mg two or three times daily or 850 mg once or twice daily with or after meals, gradually increased if necessary to 2 to 3 g daily (Sweetman, 2005). Metformin hydrochloride is slowly and incompletely absorbed from the gastrointestinal tract. The absolute bioavailability of a single 500 mg dose is reported to be about 50 to 60%, although this is reduced somewhat if taken with food. Once absorbed plasma protein binding is negligible and it is excreted unchanged in the urine. The plasma elimination half-life is reported to range from about 2 to 6 h after oral doses. Metformin is distributed into breast milk in small amounts (Scheen, 1996; Sambol *et al*., 1996).

The concentration of drug in blood is easily accessible. The pharmacological response is influenced

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by the plasma concentration of drug. It is observed that there is an optimum or desired therapeutic or pharmacological concentration range where the drug produces its characteristic effect.

Drug-drug interaction result when one drug alters the known therapeutic response of another that has been administered concurrently or before or after the drug. The next result may be enhanced or diminished effects of one or both the drugs (Hansten and Horn, 1989). A common practice in third world is the prescription of multiple drugs at a time, which may sometimes be neither safe nor effective and may be deleterious. Over the last 10 years, the research on drug-drug interactions, drug-metal interactions and drug-food interactions was carrying out by Amran *et al.* (2006a, b, 2008) and Bari *et al.* (2000). In continuation on the fate of multiple drug use the effects of gliclazide and metformin on the plasma concentration of caffeine have been studied.

The aim of present study was to evaluate the effect of gliclazide and metformin HCl on plasma concentration of caffeine and thus to infer the fate of combined drug therapies for these drugs.

MATERIALS AND METHODS

Materials: Caffeine, gliclazide and metformin HCl have been collected from the Orion Laboratories Ltd., Dhaka, Bangladesh. Disodium oxalate (reagent grade), blood serum, Na-tungstate, arsenic molybdate (reagent grade), alloxan (BDH) were used without further purification.

Equipments: UV-Visible Spectrometer (Model No. UV-1601, Shimadzu, Japan), pH Meter (Mettler Toledo, Switzerland), Power Sonic (Model No. 510, Seoul, Korea) and Dubnoff metabolic shaking incubator (GCA corporation, USA) were used in the study.

Animals: Twenty healthy rats weighing about 250±25 g were used as the experimental animals for the *in vivo* experiments. The animals were collected from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

Preparation of standard solutions: Caffeine, gliclazide and metformin HCl were dissolved in demineralized water separately. These stock solutions were diluted to desired strengths and buffered, while diluting to working solutions.

Preparation of calibration curve: For preparing a standard curve, control (1 mL) sample was taken with 1 mL of caffeine solution containing 20 μg mL^{-1} of caffeine. This was used as stock solution to prepare solutions of caffeine containing 20, 40, 60, 80,100 and 120 ng mL^{-1} of caffeine. Then absorbance was taken of each of the solution at 273 nm. Finally, absorbance values were plotted against the concentration of caffeine solutions and calibration curve was produced (Fig. 1).

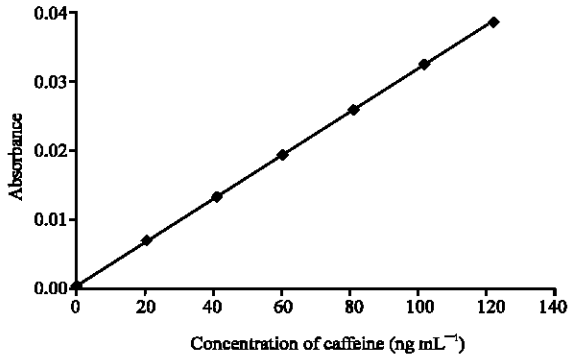

Fig. 1: Calibration curve of caffeine

Test animals and administration of drugs: Plasma samples were taken at various time intervals after a drug was orally administrated. Sixteen adult rats of 250±25 g body weight were used. They were kept rest for 7 days with normal diet. These rats were divided into 4 groups each having 4, marked as 1, 2 and 3 and one group was used as control. Here, caffeine alone and its 1:1 mixture with gliclazide and metformin HCl were administrated by orogastric tube individually in each group. They were over night fasted before drug administration. Blood samples (1 mL) were collected from cutting the tip of the tail into centrifuge tubes before drug administration and at 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 h after drug administration. All blood samples were protected from light, immediately centrifuged at 3000 rev min^{-1} for 10 min and the plasma samples were separated into vials and kept into deep freeze until taking the absorbance. The absorbance was measured at 273 nm and the plasma concentration of caffeine was determined by using calibration curve (Fig. 1) (Rahat *et al.*, 1999; Ara *et al.*, 2008).

Statistical analysis: The results were expressed as Mean ±SEM values for each experiment. Differences in mean values between experimental groups were analyzed by unpaired t-test. A probability values less than 0.05 ($p<0.05$) was defined to be significant.

RESULTS

The *in vivo* effects of gliclazide and metformin HCl on plasma concentration of caffeine have been studied by

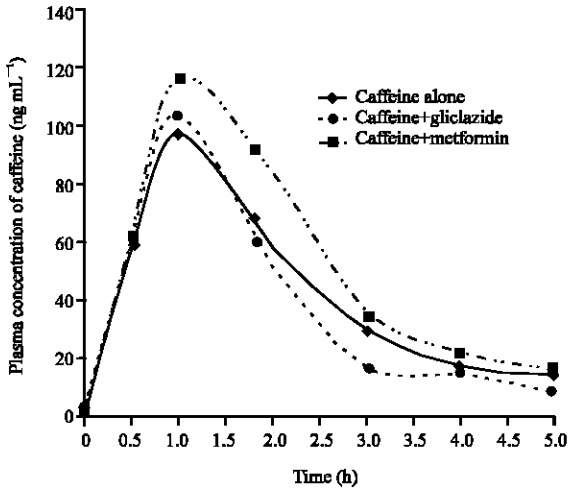

Fig. 2: Average plasma concentration of caffeine after single oral administration

observing the change in plasma concentration of caffeine in rats. In this study, the plasma concentration of caffeine was determined by UV spectroscopic method using calibration curve (Fig. 1) after oral single administration of caffeine alone and with gliclazide and metformin HCl in rats (Rahat *et al*., 1999; Bari *et al*., 2000).

From the Fig. 2, it is seen that the peak plasma concentration of caffeine is 97 ng mL^{-1}, which was obtained after 1 h of oral administration of caffeine alone.

Again, it is seen in the Fig. 2 that the oral concomitant administration of caffeine and gliclazide does not make a noticeable change in plasma concentration of caffeine. In this case the peak plasma concentration of caffeine is 104 ng mL^{-1}.

From the Fig. 2, it is also seen that the concurrent administration of caffeine and metformin HCl makes a significant change in plasma concentration of caffeine. In this case, the peak plasma concentration of caffeine is 117 ng mL^{-1}, which is significantly greater than that of caffeine when administered alone.

DISCUSSION

Concurrent administration of caffeine and gliclazide has not found any significant change in plasma concentration of caffeine. However, that of caffeine and metformin HCl showed a significant change in plasma concentration of caffeine. This may be due to higher affinity of metformin HCl for the plasma protein. So, a competitive inhibition of the binding to plasma proteins in the mixed condition can lead hazardous consequences i.e., a competitive inhibition of the binding to plasma protein by metformin HCl increases the plasma concentration of caffeine.

Such interactions of the drugs that affect the binding of plasma protein and subsequently that change the plasma concentration of the drugs are very vital to be given priority before formulating drug therapy. Since, drug displaced from plasma protein will redistribute into its full potential volume of distribution, the concentration of free drug in plasma and tissues after redistribution may be increased slightly. But this may change the pharmacokinetics properties of the drug and thereby may affect its pharmacological and toxic effects (Gilman *et al*., 1991).

Milon and Hossain (2009), Rahman and Hossain (2008) and Salam and Hossain (2001) are engaged in the study of interaction between oral anti-diabetic drugs and other agents. In such studies, most of the agents used did not interact strongly with the oral anti-diabetic agents but in the present study, metformin HCl increased the plasma concentration of caffeine. This is due to the competitive protein binding between metformin HCl and caffeine. Since, the protein binding of caffeine alone is 93% and in the presence of metformin HCl the protein binding of caffeine is 83% (Mohiuddin and Hossain, 2008).

Coffee consumption has been extensively studied in relation to various diseases, but not until recently has it been examined in relation to risk of type 2 diabetes (Van Dam and Hu, 2005). A study shows that higher coffee consumption was associated with a substantially lower risk of type 2 diabetes (Schaefer, 2004). On the other hand, this study found a significant change in plasma concentration of caffeine when metformin administered concurrently with caffeine. Therefore, the diabetic patients who are taking metformin should avoid excessive consumption of coffee or tea that contained caffeine and care and monitoring might be necessary as well.

CONCLUSION

The *in vivo* study for determination of plasma concentration of caffeine in rat by UV spectroscopic method shows that concurrent administration of caffeine and gliclazide does not make noticeable changes in plasma concentration of caffeine. But administration of caffeine and metformin HCl in rats shows a significant change in plasma concentration of caffeine. Any change in plasma concentration may affect the pharmacological or toxic effects of the drug. So, there is prospect for a combination therapy of caffeine with gliclazide or metformin HCl, particularly metformin HCl but some more *in vivo* studies are necessary to avoid any consequence of untoward incidents or harmful interactions.

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