Serum Concentrations and Hypoglycemic Effect of Gliclazide:Crosspovidone Solid Dispersion on Streptozotocin Induced Diabetic Rats

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Abstract
Gliclazide is practically insoluble in water and its GI absorption is limited by its dissolution rate. Our previously published works indicated that preparing gliclazide-crosspovidone solid dispersion in the drug/carrier ratio of 1:1 using cogrinding technique is able to enhance drug dissolution rate. The coground of gliclazide-crosspovidone was administrated to the rats and the hypoglycemic effects of pure drug, a physical mixture and the coground were considered in 3 groups of rats weighing 200–250 g (n = 6). The rats were made diabetic by single intravenous administration of streptozotocin (60 mg/kg). Each of the rats received a single dose of gliclazide (equivalent to 40 mg/kg) as pure drug, physical mixture and coground in an aqueous suspension. Glucose level was assessed via glucometer after collecting the blood samples from tail vein. Gliclazide concentration in plasma was assessed applying high pressure liquid chromatography. According to 1-way ANOVA, Student–Newman–Keuls test, the coground revealed enhanced hypoglycemic effects as well as higher serum gliclazide concentration relative to pure drug and its corresponding physical mixture in all sampling times. The area under serum glucose concentration curve vs. time for the pure gliclazide, physical mixture and coground formulations were 3090.5 ± 79, 3018.8 ± 96 and 2374.0 ± 73 mg.h/dl, respectively. Correspondingly, their area under serum gliclazide concentration curve vs. time were 1171.8 ± 156.8, 1379.5 ± 96.2 and 4827.7 ± 637.5 μg.h/ml. It follows that; formulation of gliclazide-crosspovidone coground is able to improve oral absorption of the drug.

Introduction
Gliclazide, (1-(3-azabicyclo (3.3.0) oct-3-yl)-3-(p-tolysulfonil) urea, is a second-generation hypoglycemic drug used for the treatment of non-insulin dependent diabetes mellitus, or type II diabetes. Its good general tolerability together with low incidence of hypoglycemia, designate the drug to be suitable for diabetic patients with renal impairment and also for elderly patients taking into consideration that reduced renal function may rise the risk of hypoglycemia following the administration of some sulfonlyureas [1,2]. So therefore, gliclazide could be a drug of choice in long-term sulfonylurea therapy for the control of type II diabetes. Gliclazide is absorbed slowly from the GI tract and reach the peak serum concentrations within 2–8 h after oral administration of the drug tablet [3]. Gliclazide belongs to class 2 biopharmaceutical classification system (BCS) and the slow absorption of the drug is in consequence of its poor dissolution from the formulation [4,5]. Several methods are used to enhance the dissolution rate of poorly water-soluble drugs. These methods include particle size reduction [6]; solubilization in surfactant systems [7,8]; formation of water soluble complexes [9]; drug derivatization such as strong electrolyte salt forms that commonly have a higher dissolution rate [9]; formulation of liquid-solid [10–12], decreasing crystallinity of the drug substance via formation of solid solutions [13], and solid dispersion formulations [14–16]. Solid dispersions as a most popular, simple and less expensive technique [9,17,18], improve drug aqueous solubility/dissolution rate through decrease of the drug particle size, partial transformation of the crystalline drug to the amorphous state, formation of solid solutions, formation of water soluble complexes, reduction of aggregation and agglomeration, improved wetting of the drug and solubilization of the drug.
by the carrier at the diffusion layer [19–23]. Cogrinding can be applied to improve the dissolution rate of the poorly water-soluble drugs [9, 24–28]. This method is ecologically suitable method as unlike other techniques of solid dispersion preparation it does not necessitate toxic solvents and complex equipment [9]. Our recently published works evidenced that cogrinding of gliclazide with crosspovidone in the drug to carrier ratio of 1:1 could efficiently improve the dissolution rate of the drug [9]. In the current study, solid dispersion of gliclazide with crosspovidone was prepared by cogrinding technique in the drug to carrier ratio of 1:1. Then, the hypoglycemic effects and serum concentrations of pure drug, a physical mixture and the corresponding solid dispersion were investigated on streptozotocin induced diabetic rats. To the best of our knowledge, hypoglycemic effects and serum concentrations of gliclazide-crosspovidone coground has not been investigated by the other researchers.

Materials and Methods

Materials
Gliclazide (Labching, Italy) and Glyburide (Chinoin Pharma, Hungary) were provided by Abidi Co. (Tehran, Iran). Streptozotocin (Sigma, Germany), crosspovidone (BASF, Germany), Glucose assay kit (Arkray, Japan) were used. All other chemicals used were of analytical grade.

Preparation of coground formulation and physical mixture
Unground gliclazide powder (5 g) and crosspovidone (5 g) were mixed and charged into the chamber of the vibration ball mill (Fritsch, Germany) with the drug–carrier ratios of 1:1. About half of the volume of the ball mill chamber was filled by 55 steel balls with various diameters varying 8–20 mm as well as the powder mixture. The powder mixture was subsequently ground at 360 rotations per minute for 3 h. The samples were collected, labeled and stored in a glass vial before use. The physical mixtures of drug: carrier with the ratio of 1:1 was prepared by simply blending the drug and carrier powder [9].

Streptozotocin-induced diabetes model
Diabetes was induced in the adult male rats weighing 200–250 g by intraperitoneal injection of STZ (60 mg/kg) dissolved in 0.5 ml citrate buffer (0.1 M, pH = 4.6). The permission for animal studies was obtained from the ethics committee of Tabriz University of Medical Sciences. All animal experiments were also carried out in compliance with the “Guide for the Care and Use of Laboratory animals” of Tabriz University of Medical Sciences. Fasting blood glucose levels were measured 48 h after STZ injection and rats with blood glucose levels over 200 mg/dl were classified as diabetic.

Serum glucose concentration
The diabetic rats were randomly divided into 3 groups (6 rats/group). Each of the rats received a single dose of gliclazide (equivalent to 40 mg/kg) as pure drug (group A), physical mixture of drug (group B) and solid dispersion (group C) in an aqueous suspension. The suspensions were prepared by dispersing the pure drug powder or its solid dispersion and physical mixture in 10 ml distilled water and administered in the esophagus with a syringe equipped with a number 12 catheter. The animals were fasted 12 h before the experiment, with free access to water. Blood samples were collected predose (0h) and 1, 3, 6 and 8h post dose from tail vein. Considering that, in the ascending part of the drug serum concentration curve, the absorption rate is dominant compared to elimination rate and the dissolution rate is the major factor affecting the serum concentrations, attempts were not made to collect the blood samples beyond 8 h. Glucometer (Arkray, Japan) was used to analysis of serum glucose concentration from the collected blood samples. In order to analysis of drug serum concentration, the blood samples centrifuged at 3 500 rpm for 15 min, and the serums were collected and kept at −20 °C.

Gliclazide serum concentration
Gliclazide concentration in plasma was measured by an established HPLC method [29, 30] with slight modification. Briefly, 10 μL of glyburide (1 mg/mL) as an internal standard was added to 100 μL of plasma sample and vortex-mixed for 10s. After that, 200 μL of acetonitrile was added to the mixture and was vortex-mixed for 10s once again and followed by centrifugation at 13 000 rpm for 3 min. The dried residue of the acetonitrile extract obtained under vacuum at 40 °C was redissolved in 100 μL K2HPO4 (pH = 4.6)-acetonitrile (30:70 v/v) as a mobile phase, and a 20 μL aliquot was injected into the HPLC system. The mobile phase was delivered at the flow rate of 1 mL/min and separation was achieved on a C18 column (4.6 × 250 mm, 5 μM Analysentechnik GmbH, Mainz, Germany). Gliclazide was detected at 229 nm wavelength with an ultraviolet detector. Chromatographic data was evaluated applying Data Control program supplied with the HPLC system. A linear calibration plot prepared using peak height of the standard solutions (0.0125–0.1 mg/mL) of gliclazide was employed to analyze the concentration of the drug in the serum samples.

Statistical analysis
1-way ANOVA, Student–Newman–Keuls method was conducted to compare the mean drug serum concentrations at different times for 3 different treatments [31]. Data was represented as mean values ± SEM (standard error of means). A p-value less than 0.05 was assumed for the statistically significant differences.

Results

Serum glucose concentration
Fig. 1 demonstrates the effects of pure gliclazide, physical mixture and coground formulations on serum glucose concentrations at different times. Statistical analysis divulges that the serum glucose concentrations following administration of the coground were significantly lower (p < 0.001) than those of pure gliclazide and physical mixture in all the times; however, there was no significant difference (p > 0.05) between the latter 2 formulations (Fig. 1). The area under serum glucose concentration curve vs. time (AUCg0 8) for the pure gliclazide, physical mixture and coground formulations were 3 090.5 ± 79, 3 018.8 ± 96 and 2 374.0 ± 73 mg.h/dl, respectively. The AUCg0 8 value for the coground was significantly (p < 0.001) higher than the corresponding values for pure gliclazide and physical mixture; whereas there was no significant difference (p > 0.05) between the AUCg0 8 values of pure gliclazide and physical mixture.
Gliclazide serum concentration

Gliclazide serum concentration was measured applying an established HPLC assay with a minor modification [32, 33]. The retention times for gliclazide and glyburide, used as an internal standard, were 6 and 8 min, respectively. The regression equation for the calibration curve which was obtained by plotting the peak height ratio vs. concentration was as follow:

\[ y = 0.0961x + 0.0042 \quad (r^2 = 0.9948) \]

The mean serum concentrations of gliclazide following a single oral dose (40 mg/kg) of pure gliclazide, physical mixture as well as coground at the different times are shown in Fig. 2. As in Fig. 2, the serum drug concentrations after administration of the coground were statistically (p<0.001) higher than those of pure gliclazide, physical mixture in all the sampling times; while there was no statistical difference (p>0.05) between 2 later preparations.

Discussion

Our previously published works revealed that the cogrinding method can be employed to enhance the dissolution rate of the poorly water-soluble drug, gliclazide [9]. Our published results pointed out that the coground of gliclazide with crosspovidone in the ratio of 1:1 could improve the drug dissolution rate, efficiently [9]. So therefore, in the current study, the mentioned formulation was prepared to investigate the in vivo oral absorption of the solid dispersion in the rats. Administration of the coground could reduce serum glucose concentrations significantly more efficient (p<0.001) than pure gliclazide and physical mixture. The difference in serum glucose concentrations could be explained by corresponding difference in the dissolution profiles, explicitly, the higher dissolution rate lead to the lower serum glucose concentration. The higher dissolution rate can be accounted for the higher hypoglycemic effect of the coground formulation as well. The drug serum concentrations next to coground administration were statistically (p<0.001) higher than the other preparations. The results were in accordance with serum glucose concentration data indicating that oral absorption of gliclazide enhances after its administration as a coground system with crosspovidone which could be due to enhanced dissolution rate of drug in the coground formulation. Similar results have been reported by Asayrie S., et al. for the solid dispersion of gliclazide with PEG 6000 [34]. Although, precise estimate of pharmacokinetic parameters was not possible because of insufficient plasma sampling data, but it is evident in Fig. 2 that the coground of gliclazide with crosspovidone resulted in considerably higher C\text{max} (the peak plasma concentration) as well as lower T\text{max} (the time required to reach the peak) in comparison with pure drug and physical mixture.

Conclusion

As indicated by this study, formulation of gliclazide-crosspovidone solid dispersion directing cogrinding method is able to improve oral absorption of the drug. That is, preparation of gliclazide-crosspovidone coground resulted in elevation of drug serum concentration and decrease of serum glucose concentration. The higher dissolution rate of the coground compared to pure gliclazide and its physical mixture with crosspovidone can be accounted for the higher hypoglycemic effect of the coground formulation.

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Conflict of Interest

The authors report no conflicts of interest.

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