Original article

Assessment of glibenclamide pharmacokinetics in poloxamer 407-induced hyperlipidemic rats

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A B S T R A C T

The aim of the present research was to describe the consequences of hyperlipidemia (HL) on the pharmacokinetics of glibenclamide (Gb) in poloxamer 407-induced hyperlipidemic rats. Rats were given intraperitoneal dose of poloxamer 407 to cause hyperlipidemia. A single oral dose of Gb (10 mg/Kg) was given to normal and HL rats. The C_{max} and t_{max} after oral dose of Gb in normal rats were 340.10 μg/ml and 3.67 h, respectively. Whereas, the C_{max} and t_{max} after oral dose of Gb in HL rats were noted as 773.39 μg/ml and 2.50 h respectively. The AUC value of Gb was found considerably higher in the HL rats. While the plasma clearance (CL) after oral dose of Gb was 2.53 ml/h and 1.39 ml/h in normal and HL rats respectively. The improved plasma concentration of Gb following oral dosing in rats with HL seems to be due to a direct influence on hepatic clearance or metabolizing enzymes. In conclusion, the Gb pharmacokinetics was considerably affected by the HL in rats. Such findings play an important role for predicting the alterations in the pharmacokinetics of drugs including GB, in cases having hyperlipidemia.
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1. Introduction

Hyperlipidemia (HL) is a clinical condition that is defined by rise in plasma lipoproteins. This situation presents a clinical concern, because chronic rises in low-density lipoproteins are a key factor contributing to an increased risk of coronary heart disease, atherosclerosis and hypertension. Previous reports have reported that HL could contributes certain changes in the toxicodynamic, pharmacodynamic and pharmacokinetic profiles of lipoprotein-bound drugs (Hamdy and Brocks, 2009, 2016; Khalil et al., 2016; Lee et al., 2019). HL might be associated with increased plasma binding and a lower unbound plasma fraction, with a decrease in total drug volume of distribution (Vd) and likely reduce the clearance (CL) of the drug. In general, decreased in clearance of drug leads to increase the concentration of drug in systemic circulation

to toxic level and that apparently contribute to increased potential for adverse drug reactions. Conversely, increased in clearance of drug contributes to lesser side effect of drug. However, in cases where drug clearance is greater, higher weight-based doses and more frequent dosing intervals are needed. Hence, when it comes to medication dosing, knowing the importance of drug clearance is vital. Drug dosages and their frequency of administration must also be adjusted for cases with HL in attempt to avoid undesirable drug effects (Malangu, 2018).

HL has also been associated with a decreased level of expression and some activity of cytochrome P450 (CYP) isoforms that are primarily engaged in drug metabolism (Patel and Brocks, 2009). Antecedently, HL has been shown to modify the pharmacokinetic profile of several candidate in rats (Khalil et al., 2017; Khalil et al., 2016; Lee et al., 2012; Lee et al., 2019; Bin Jardan and Brocks, 2016).

Glibenclamide (Gb, Fig. 1) is an oral 2nd generation sulfonylurea, with a potent and sustained hypoglycemic impact, often prescribed to manage type 2 diabetes mellitus (Ju et al., 2020; Zou et al., 2020). Gb is also widely considered to treat gestational diabetes mellitus (Affres et al., 2020; Bouchghoul et al., 2020; Shuster et al., 2020), Gb has been observed to accumulate in pancreatic beta cells after chronic use (Hellman et al., 1984). These variables can lead to the extended action of the drug and cause hypo-

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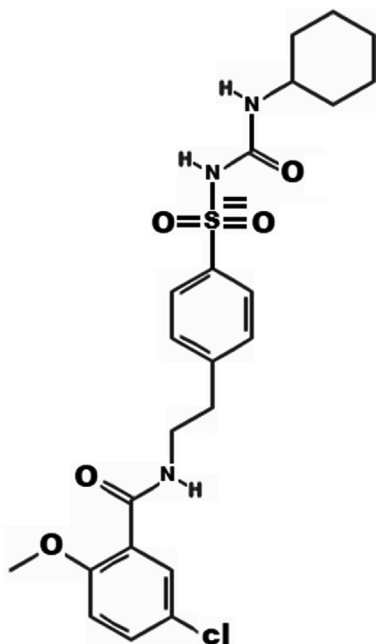Peer review under responsibility of King Saud University.

Fig. 1. Chemical structure of Gb.

glycemia in certain circumstances. Gb is extensively metabolized in the liver through the CYP system (Naritomi et al., 2004; Zharikova et al., 2009; Zhou et al., 2010). Several studies have shown that the main CYP enzyme concerned in the *in vitro* metabolism of Gb is CYP3A4 (Zhou et al., 2010). Although, other CYPs, for instance, CYP2C8, CYP2C9 and CYP2C19 could also play a role in Gb *in vitro* metabolism (Zharikova et al., 2009; Zhou et al., 2010). The pharmacokinetics of Gb and its excretion in hyperlipidemia condition is unclear. Gb is sparingly soluble in water and categorized in the list of BCS class II drugs (Ahad et al., 2015) and having log p of 4.7 (Spiller, 2014). Therefore, Gb is a possible candidate for lipoprotein binding and therefore might show potential changes in its pharmacokinetics. Consequently, the objective of this research was to examine the potential effects of raised lipoprotein levels on the Gb pharmacokinetics in the poloxamer induced - HL rats.

2. Materials and methods

2.1. Materials

"Glibenclamide was purchased from Alfa Aesar (Ward Hill, MA)". Poloxamer-407 was purchased from "Anatrace Products, LLC Maumee, Ohio, USA". HPLC grade methanol and acetonitrile were procured from "Panreac Quimica (Barcelona, Espana)". Formic acid was acquired from "Loba Chemie Pvt. Ltd. (Mumbai, India)". "Purified water was arranged using Milli-QR Gradient A10R ("Millipore, Molsheim Cedex, France").

2.2. Animals and induction of HL

Experimental protocols were duly approved by the "King Saud University Research Ethics Committee with ethical reference number KSU-SE-21-10". Wistar rats (250 ± 20 g) were used in this study. All rats were housed in temperature-controlled rooms with 12 h of light per day. Rats were distributed into two groups and named as NL (control group, *n* = 3) and HL (*n* = 4) groups. HL was induced by single intraperitoneal injection of 1 g/kg of Poloxamer-407. Control and the HL groups were dosed with the

drug; HL group were treated after 36 h from the P407 injection (Chaudhary and Brocks, 2013; Khalil et al., 2017; Khalil et al., 2016).

2.3. Drug administration and sampling

For the pharmacokinetic study, the both groups of rats received Gb 10 mg/kg via oral feeding needle. Blood samples were taken in heparinized tubes from NL and HL groups at the time interval of 0, 0.5, 1, 2, 3, 4, 12, 24 h after the Gb oral dose. The plasma was separated by centrifugation of the blood at 13,000 rpm for 10 min and transferred to clean eppendorf tubes. The separated plasma samples were analyzed using a UPLC-MS/MS method. Briefly, plasma protein precipitation method was used to prepare the samples. As an internal standard, glimepiride was included. An Acquity UPLC®BEH C_{18} column was used for the analysis. A mixture of acetonitrile (0.1% formic acid) and water (0.1% formic acid) was used as mobile phase which was pumped at a flow rate of 150 μl/min in binary gradient mode. Positive electrospray ionization was used to operate the triple- quadrupole mass spectrometer. In MRM mode, sodium adducts $[M+Na]^+$ of Gb and internal standard were observed. The sample 10 μl was injected and the autosampler temperature was maintained at 20 ± 3 °C. Total sample run time was 2.0 min (Alam et al., 2018). The non-compartmental pharmacokinetic parameters were estimated using PK Solver software (version 1.0). The calculated parameters were as follows: C_{max}, maximum plasma concentration; T_{max}, time to reach maximum plasma concentration; AUC_{0-t}, area under the plasma concentration–time curve; $t_{1/2}$, elimination half-life. The CL, total clearance was calculated as dose/AUC.

2.4. Statistical analysis

"Differences in means were analyzed by unpaired *t*-test; using GraphPad InStat® 3.06 (GraphPad Software, Inc, CA, USA). p < 0.05 were considered significant".

3. Results and discussion

A single dose of poloxamer-407 caused HL in rats; this model is an acceptable choice to evaluate the effect of HL in the pharmacokinetics of drugs, since it is non-inflammatory and not linked with diabetes or cardiovascular disruption (Gabr et al., 2017).

In different animal models, poloxamer-407 can stimulate hyperlipidemia, which greatly enhances the triglycerides and cholesterol in plasma (Johnston and Palmer, 1993; Kim et al., 2019; Palmer et al., 1998; Wout et al., 1992; Yeom et al., 2018). Antecedently, the pharmacokinetics of several medications, including amiodarone, docetaxel, nifedipine, and cyclosporine A, have been shown to substantially changed in hyperlipidemia. A marked increase in plasma amiodarone concentrations and reductions in clearance, were documented in hyperlipidemic rats (Shayeganpour et al., 2005). In another study, the unbound fraction and intrinsic liver metabolism of docetaxel were considerably lowered; this could be owing to the lower expression of CYP3A (Lee et al., 2011). In another study, the clearance of nifedipine in hyperlipidemic rats was slightly lower owing to the decline in unbound fraction in plasma (Eliot et al., 1999). On the other hand, the CL of cyclosporine A was not altered in hyperlipidemic rats, but the unbound plasma fraction, and Vd, were substantially reduced (Brocks et al., 2006).

Based on above literature, the pharmacokinetics of Gb, which has a lipophilic property and is metabolized by CYP3A, could also be modified in the hyperlipidemic state. The Gb plasma concentration–time profiles in normolipidemic (NL) and HL rats after oral
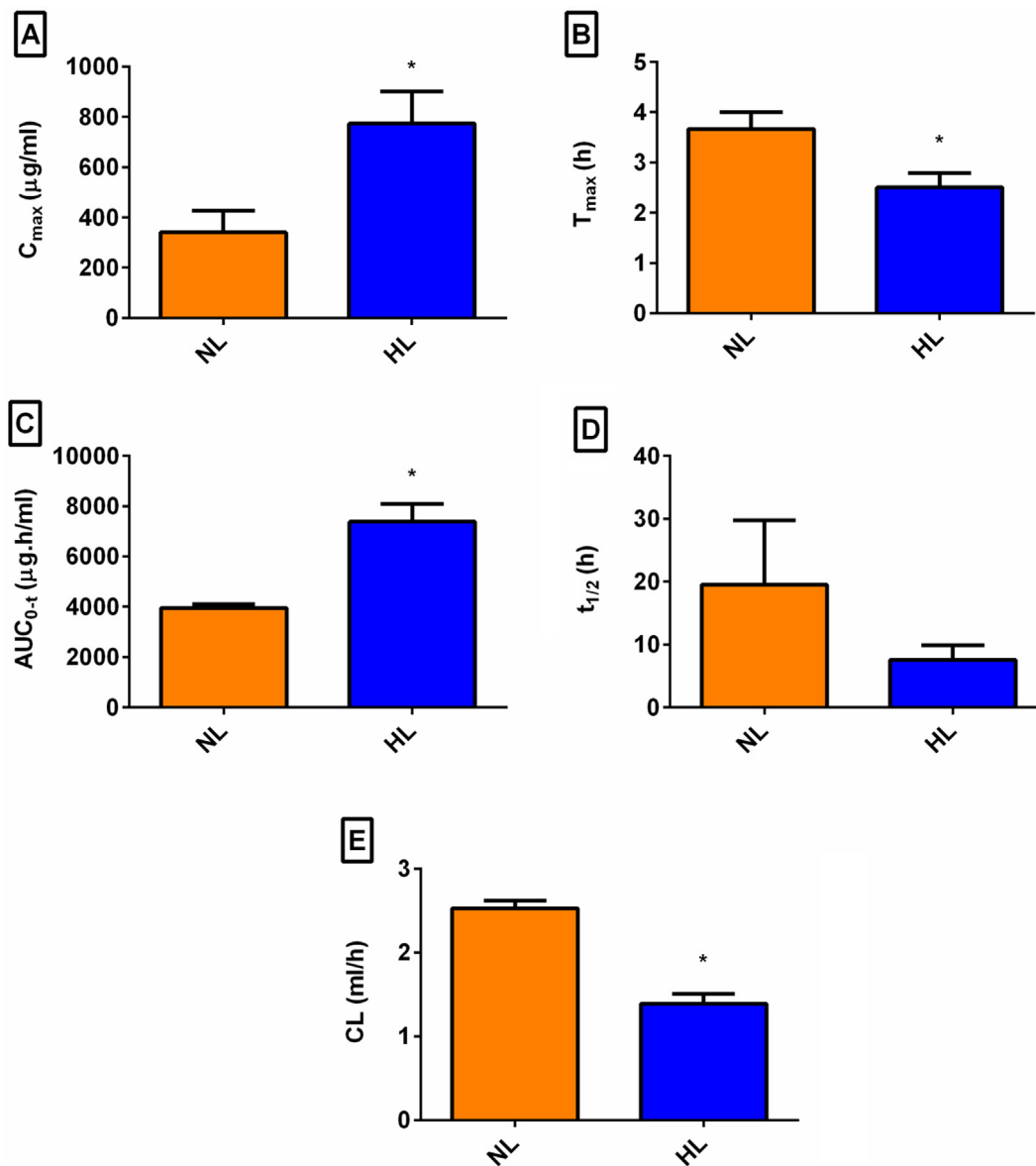
Fig. 3. Pharmacokinetic parameters of Gb such as (A) C_{max}, (B) T_{max}, (C) AUC_{0-t} and (D) $t_{1/2}$, and (E) CL, in NL and HL rats.
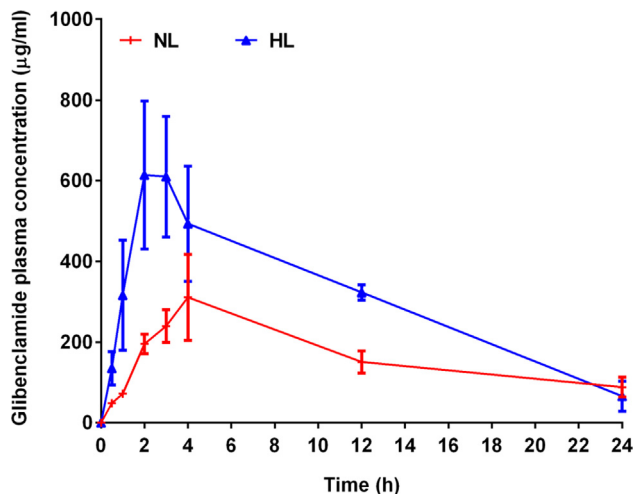

Fig. 2. Gb plasma concentration verses time profile in NL and HL rats.

treatment are illustrated in Fig. 2, and the estimated pharmacokinetic parameters are displayed by bar graphs in Fig. 3.

In this study, a single dose of poloxamer-407 successfully caused HL in rats. It was observed that the blood lipid levels of treated rats (data not shown) were considerably higher than those of the control group, suggesting that the hyperlipidemia rat model has been successfully established. In present study, the C_{max} of Gb in NL group was found to be 340.10 μg/ml, whereas the C_{max} of Gb in HL group was found to be 773.39 μg/ml. There is an about 127% increase in C_{max} of Gb was observed in HL group. On the other hand, the T_{max} of Gb in HL rats was decreased by 32%, the T_{max} of Gb in NL rats was found to be 3.67 h, whereas the T_{max} of Gb was little shifted to earlier time 2.50 h in HL rats. The AUC_{0-t} of Gb in NL rats was found to be 3956.21 μg.h/ml, interestingly the AUC_{0-t} of Gb in HL rats was increased by 87% and reached to 7380.38 μg.h/ml. On other side, the CL of Gb was deceased by 45% (Fig. 3). The $t_{1/2}$ of Gb in NL rats was calculated as 19.51 h while the $t_{1/2}$ in HL rats was noted as 7.59 h, although the difference was not significant. The results of present study are in agreement with the previous findings that reported lesser $t_{1/2}$ of

investigated drug in HL rats (Katnapally et al., 2009; Khalil et al., 2017; Khalil et al., 2016). The CL of Gb in NL rats was recorded as 2.53 ml/h while the CL in HL rats was noted as 1.39 ml/h. Clearly, the findings of present study demonstrated better pharmacokinetics parameters such as C_{max}, and AUC_{0-t} of Gb in poloxamer 407-induced hyperlipidemic rats as compared to control group rats. While the T_{max} and CL of Gb was decreased in hyperlipidemic rats as compared to control group rats. It was observed here that the bioavailability of Gb was found to increase by 1.88 fold in rats with HL. Based on the previous reports, it concluded that reduced hepatic clearance of Gb alone could not cause an increase in AUC_{0-t} value in HL rats after oral administration. It was reported that in HL rats, lowered intestinal metabolism associated by reduced hepatic metabolism of Gb following oral administration could be the reason of the higher AUC_{0-t} value (Lee et al., 2012). It was reported that the overall CYP content was markedly lower in the liver microsomal protein of HL rats than in control rats (Shayeganpour et al., 2008). Investigators presented noteworthy reductions in the protein expressions of CYP2C11, CYP3A1, and CYP3A2 in HL rat hepatic microsomes as compared to control rats. The GB is extensively metabolized by hepatic enzymes, hence this could be postulated that such low clearance of Gb in the HL rats could be due to the down regulation of hepatic CYP3A1/2; when the 73% homology among the human CYP3A4 and rat CYP3A1 protein was taken into consideration (Gay et al., 2010; Guengerich et al., 2016; Manikandan and Nagini, 2018).

4. Conclusion

In summary, the consequences of HL in rats were examined using Poloxamer 407 model. This method has been reported to provide a substantial impact on the pharmacokinetics of several drugs. In this study, following oral administration, the AUC value of Gb was substantially greater in the HL rats, this could be due to the decreased hepatic and intestinal metabolism of Gb. These observations have important role for predicting the alterations in the pharmacokinetics of drugs including GB, in cases having hyperlipidemia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

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