Pharmacokinetic changes of halofantrine in experimentally-induced diabetes mellitus following oral drug administration

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It was hypothesized in this study that alterations in plasma lipoprotein profile and disturbed gastrointestinal motility as observed in diabetes mellitus may influence the disposition of halofantrine (HF), a highly lipophilic antimalarial drug. Therefore, using a rat model of diabetes mellitus induced by administration of alloxan monohydrate, the effects of the disease on the pharmacokinetics of HF was investigated. Also, the drug binding to normal and diabetic plasma components was determined. Results showed that the mean $C_{\text{max}}$ values of HF and its major metabolite, desbutylhalofantrine (DHF), were markedly higher (up to 2.5 times) in the control than in diabetic rats ($p < 0.05$). Also, the early AUC ($AUC_{0-12}$) and rate of drug absorption ($C_{\text{max}} / AUC_{0-\infty}$) were markedly reduced by 40 and 58\%, respectively, in diabetic compared to control group. However, the $T_{\text{max}}$, $AUC_{0-\infty}$, and elimination $T_{1/2}$ of HF were comparable between the two groups ($p > 0.05$). The binding of HF and DHF in diabetic plasma was significantly higher when compared to control ($p < 0.05$) and correlated well with increased triglycerides concentrations. Elevated plasma drug levels expected in diabetes due to observed marked increase in drug binding to plasma components appear to be counterbalanced by other pharmacokinetic-modulating processes induced by the disease. It is suggested that the significantly reduced $C_{\text{max}}$ of the drug and its metabolite in diabetes may have clinical implications since the clinical efficacy of HF is influenced by its peak plasma concentrations.

Key words: Halofantrine, pharmacokinetics, drug plasma binding, lipoproteins, diabetes mellitus.

INTRODUCTION

Halofantrine (HF) is a highly lipophilic antimalarial drug which is effective against multidrug resistant strains of \textit{P. falciparum} (Boudreau 1988). The occurrence of malaria with other long term diseases such as cardiovascular and metabolic disorders is common. One of such metabolic disorders is diabetes mellitus (Wändell et al., 1996) which is a heterogeneous clinical syndrome associated with alterations in protein, lipoprotein and free fatty acid plasma concentrations (Gwilt et al., 1991; O'Connor and Feely, 1987). These alterations influence the pharmacokinetics of some drugs (Goldstein et al., 1990; Zysset and Sommer, 1986). For example, increased plasma levels of triglycerides-rich lipoproteins are usually observed in diabetes mellitus and these result from decrease in the activity of lipoprotein lipase (Nikkil, 1973). Like many other highly lipophilic drugs, HF binds significantly to lipoproteins (Urien, 1986; Florence and Halbert, 1991;  

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Investigation of the binding of HF with various plasma lipoprotein fractions showed that HF binds significantly to triglyceride-rich lipoproteins and that the clearance of HF is influenced by plasma lipoprotein profiles (Humberstone et al., 1998). Thus, since HF binds significantly to lipoproteins, elevated plasma lipoprotein concentrations observed in diabetes mellitus may significantly alter the disposition of the drug.

Several authors have reported altered activities of different cytochrome P450 isoforms, and changes in the glomerular filtration rate in rats with experimental diabetes or in humans presenting with diabetes mellitus (Zysset and Tlach, 1987; Mahachai et al., 1988; Goldstein et al., 1990; Shimojo et al., 1993). Studies have shown that there is an increase in the expression and mRNA levels of CYP3A1(23) in rats with diabetes mellitus induced by Alloxan or Streptozotocin (Raza et al., 1996; Lee et al., 2004; Kim et al., 2005). Since HF is mainly metabolized in humans by CYP3A4 (Halliday et al., 1995), a homologous human form of CYP3A1(23), an alteration in the pharmacokinetics of the drug in diabetes mellitus can also be mediated through increased activity of the enzyme induced by the disease. In addition, gastrointestinal symptoms such as nausea, vomiting, diarrhea, constipation, and fecal incontinence are often encountered in patients with diabetes (Schvarcz et al., 1996; Spanggys et al., 1999). These symptoms have been attributed to disturbed gastrointestinal motility resulting in delay in gastric emptying. Hence, diabetes can affect the absorption of orally administered drugs.

Rats and dogs have been used as models for the study of the absorption characteristics of HF (Porter et al., 1996; Humberstone et al., 1996; Brocks, 2002) as well as for investigation of effects of diabetes mellitus induced by Alloxan on drug disposition. The pharmacokinetic properties of HF enantiomers in the rat related to those observed in humans, indicating that rat is a good model for the study of halofantrine pharmacokinetics (Brocks and Toni, 1999).

From the foregoing, there is a theoretical possibility that diabetes mellitus can modulate the pharmacokinetics of HF. Therefore, using rat model of experimental diabetes mellitus, this study sought to investigate the influence of the disease on the disposition of HF, and also to elucidate the contribution of plasma protein binding to any observed changes in the drug pharmacokinetics induced by diabetes.

MATERIALS AND METHODS

Animals and animal care

White Albino Swiss rats of both sexes weighing 150 – 250 g obtained from the department of Pharmacology, Obafemi Awolowo University, Ile Ife, Nigeria, were used for the study, and the protocol was approved by the Animal Care Committee of the institution. All the animals were cared for in clean cages and supplied with standard rats feed and water ad libitum. The animals were used and cared for in accordance with the guidelines and procedures contained in the “Principle of laboratory animal care” (NIH publication No. 85-23, revised 2002).

Chemicals and reagents

Powders of halofantrine hydrochloride and desbutylhalofantrine were obtained from Smithkline Beecham Pharmaceuticals, Welwyn UK. Sodium dihydrogen phosphate, potassium dihydrogen phosphate, sodium chloride (all from BDH Poole, UK) and sodium hydroxide (Sigma, St. Louis, USA) were used in preparing solutions. Acetonitrile (Aldrich, England, UK) and Methanol (Chromasol®) were of high performance liquid chromatography (HPLC) grade and were obtained from Sigma - Aldrich, Diethyl ether (BDH Poole, UK) and n-hexane (BDH Poole, UK) were of analytical grade. Perchloric acid (H and W Essex, UK) was used in mobile phase preparation. Alloxan monohydrate was obtained from Sigma - Aldrich. Randox® diagnostic reagent kits were used for biochemical analyses of plasma samples for bilirubin, cholesterol and triglyceride and Bovine Serum Albumin (BSA) was used as standard in protein analyses.

Induction of diabetes

Diabetes mellitus was induced by a single intraperitoneal administration of 150 mg/kg alloxan monohydrate and followed by being allowed a 5% (w/v) glucose drink for 24 h. Diabetes was confirmed on day 12 post alloxan using blood glucose test strips (ACCU CHEK® Roche) and a glucometer (Glucotrend® Roche, Serial No. GH02679890). Blood was collected via the tail vein and animals with blood glucose readings equal to or greater than 11 mmol L⁻¹ (200 mg dl⁻¹) were included in the study. Up to 90% of the animals subjected to this treatment achieved the blood glucose level cut-off criteria.

Drug administration and sample collection

Rats were randomly divided into two groups – control and diabetes – having 48 rats per group. Each group was further subdivided into 12 subgroups of 4 rats each, with each subgroup representing a time point for blood sample collection. Diabetes was induced as described above. All the animals were weighed on days 1 and 12 following administration of alloxan. The control group received the same treatments as the diabetic group except that normal saline was administered in place of alloxan.

On day 12, each animal was given an oral dose of 8 mg/kg halofantrine hydrochloride suspension (Halfan®, Smithkline Beecham, Lagos, Nigeria) using oral cannula, except for zero hour subgroup that received 0.5 ml of distilled water. Prior to drug administration, food was withheld overnight and restored at least 2 hours after dosing. Thereafter, animals were anaesthetized with halothane and blood samples were collected by cardiac puncture and placed in EDTA tubes at 0 (without halofantrine dose) and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h following the administration of the drug. The blood samples were centrifuged at 2000 g for 15 min.

Plasma protein binding studies

In vitro plasma protein binding of HF and DHF in control and diabetic groups was determined using erythrocyte vs buffer or diluted
plasma partitioning method (Schulmacher et al., 2000; Brocks 2002). In brief, rats were anaesthetized and blood was collected by cardiac puncture and transferred into heparinized tubes. Blood from each rat was split into two different tubes and plasma was separated from the blood cell by centrifugation at 2000 g for 10 min. The blood cell obtained after removal of the buffy coat layer were washed in an equal volume of isotonic potassium phosphate buffer (pH = 7.4) containing 1.41 g/l KH$_2$PO$_4$, 0.26 g/l NaH$_2$PO$_4$, and 8.10 g/l NaCl. The washing procedure was performed three times and each washing was followed by centrifugation. The final volume of erythrocytes in each of the tubes was measured and either the isotonic buffer or diluted plasma was added to achieve a hematocrit of 0.3 for buffer or 0.4 for diluted plasma. The diluted plasma was a 1 in 20 dilution of the pooled plasma with isotonic potassium phosphate buffer.

Stock solutions of HF.HCl or DHF were spiked into diluted plasma- or buffer-containing erythrocytes mixtures to obtain final concentrations of approximately 25 µg/ml. Seven incubations each of the paired erythrocytes suspension were made for HF and DHF separately for both control and diabetic rats at room temperature in a laboratory shaker for 1 h. This duration of incubation had been established as long enough for equilibration (Brocks, 2002). The erythrocytes were separated from the aqueous phase by centrifuging at 2000 g for 10 min and the aqueous layer transferred into another tube and stored frozen at -20°C until drug analyses.

Drug analyses

Plasma samples from rats in the pharmacokinetics study, and the aqueous phase (diluted plasma or buffer) from the plasma protein binding study were analyzed for HF and DHF using a validated previously reported HPLC method (Onyeji and Aideloje, 1997) as modified by Omoruyi et al. (2007). This is a nonstereospecific assay used for the quantification of halofantrine and its metabolites (DHF). The modification involved the use of Diazepam (10 µl of 10 µgml$^{-1}$ solution in 1 ml plasma) as the internal standard instead of the chloro-substituted derivative of halofantrine (Onyeji and Aideloje, 1997). The HPLC equipment consisted of an Agilent 1100 HPLC system fitted with a quaternary pump with an online vacuum degasser and 1100 Diode Array Detector (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was achieved with an Eclipse XDB-C8 analytical HPLC column (5 µm particle size and 150 x 4.6 mm, i.d.) and detector wavelength was set at 254 nm. The mobile phase consisted of Methanol: 0.05 M NaH$_2$PO$_4$ (73:27, v/v) with 55 mM perchloric acid. The pH of mobile phase was adjusted to pH 3.0 and pumped through the column at ambient temperature and a flow rate of 1 ml/min. Retention times of the internal standard, DHF and HF were 3, 7 and 10.5 min, respectively. The limits of detection were 2.5 and 2.0 ng ml$^{-1}$ for HF and DHF, respectively. The intra-day and inter-day assay coefficients of variation for both compounds were less than 8% at concentrations of 100 and 800 ng ml$^{-1}$ (n = 6), while the absolute recovery from the plasma (normal and diabetic plasma) at the corresponding concentrations was not less than 90%, respectively.

Biochemical assays

Biochemical assays of the plasma samples from rats in the pharmacokinetics study were performed for total bilirubin, cholesterol and triglyceride by established methods using Randox$^{TM}$ diagnostic reagent kits based on assay techniques described earlier (Jendrassik and Grof, 1938; Trinder, 1969). Protein concentration was estimated by Lowry assay techniques with its reported modifications (Lowry et al., 1951; Hartree 1972; Dulley and Grieve, 1975; Bensadoun and Weinstein, 1976). Standard calibration curve for protein was obtained using bovine serum albumin (BSA) and the graph was linear over the concentration range of 16 – 80 µg mL$^{-1}$, having correlation coefficient (r$^2$) value of 0.9908.

Data analysis

The rats in each of the 12 subgroup representing a time point were randomly assigned numbers i – iv and, rats with the same number were used to generate drug concentration vs time profiles in the pharmacokinetics study. From the four generated concentration vs time profiles, the peak plasma concentrations ($C_{max}$) and the time to reach peak concentration ($t_{max}$) were obtained directly. Other pharmacokinetic parameters such as area under the plasma concentration time curve (AUC), terminal elimination half-life ($T_{1/2p}$), and Mean Residence Time (MRT) were calculated using standard noncompartmental methods (Gibaldi and Perrier, 1982) with the WinNonlin Standard Edition, Version 1.5 software package (Scientific Consultant Inc. Apex, NC, USA).

The calculations of the unbound fractions (fu) of the drugs in plasma were determined using a series of equations outlined by Schulmacher et al. (2000). The erythrocyte concentration of HF or DHF in the erythrocyte-diluted plasma ($C_E$) was determined by the following equation:

$$C_E = [C_b - C_0 (1 - HCT)] / HCT$$

Where $C_b$ is the known concentration of the drug in the erythrocyte – diluted plasma suspension, $C_0$ is the determined concentration of the drug in diluted plasma, and HCT is the hematocrit in the erythrocyte – diluted plasma sample.

To determine the erythrocyte concentration of the drug in the erythrocyte – buffer samples ($C_{E*}$), the Eq.1 was used but $C_b$ represents the known concentration of the drug in the erythrocyte – buffer suspension, and the determined buffer concentration of the drug ($C_b$) was substituted for $C_p$ while HCT is the hematocrit in the erythrocyte – buffer sample. It was noted that the HCT (volume ratio of blood cells in the suspension) were 0.4 and 0.3 for erythrocyte-plasma suspension and erythrocyte-buffer suspension, respectively. The unbound fraction, fu, of the drugs was determined by:

$$fu = (\alpha P_f / P_b) / [1 - (P_f / P_b x (1 - \alpha))]$$

Where $\alpha$ is the plasma dilution factor and with a 20-fold dilution of the plasma used, the value was 0.05. $P_f$ and $P_b$ represent the partition coefficients for the erythrocyte:diluted plasma or erythrocyte:buffer, respectively, and these were determined from the ratios: $C_{E*} / C_p$ and $C_{E*} / C_b$, respectively.

Statistical analysis of the data was performed using the Student’s t-test for paired or unpaired observations as applicable. Pearson correlation coefficient was used to evaluate the relationship between pairs of data. A p value < 0.05 was considered to be statistically significant.

RESULTS

The mean ± standard deviation (SD) of the weights of diabetic rats and blood glucose levels at day 1 and 12 following alloxan administration are presented in Table 1 along with the weights of the control rats that received normal saline in place of alloxan at the corresponding days. The weights of the diabetic rats markedly decreased (p < 0.05) while the blood glucose levels showed a
Table 1. Average weights of control and diabetic rats and average blood glucose levels in diabetic rats on days 1 and 12, following administration of normal saline (for control) or alloxan (for diabetic) (mean ± SD, n = 4).

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Control rats weights (g)</th>
<th>Diabetic rats weights (g)</th>
<th>Diabetic rats glucose (mmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 12*</td>
<td>Day 1</td>
</tr>
<tr>
<td>1</td>
<td>200 ± 20.4</td>
<td>219 ± 12.5</td>
<td>208 ± 35.4</td>
</tr>
<tr>
<td>2</td>
<td>169 ± 23.8</td>
<td>191 ± 18.8</td>
<td>231 ± 25.0</td>
</tr>
<tr>
<td>3</td>
<td>181 ± 37.6</td>
<td>203 ± 41.4</td>
<td>206 ± 12.5</td>
</tr>
<tr>
<td>4</td>
<td>206 ± 12.5</td>
<td>222 ± 21.4</td>
<td>181 ± 31.4</td>
</tr>
<tr>
<td>5</td>
<td>181 ± 31.4</td>
<td>200 ± 33.8</td>
<td>163 ± 12.5</td>
</tr>
<tr>
<td>6</td>
<td>187 ± 25.0</td>
<td>209 ± 23.6</td>
<td>206 ± 25.0</td>
</tr>
<tr>
<td>7</td>
<td>169 ± 37.6</td>
<td>188 ± 25.0</td>
<td>169 ± 24.0</td>
</tr>
<tr>
<td>8</td>
<td>191 ± 11.9</td>
<td>219 ± 12.5</td>
<td>169 ± 12.5</td>
</tr>
<tr>
<td>9</td>
<td>191 ± 27.8</td>
<td>216 ± 11.9</td>
<td>175 ± 10.2</td>
</tr>
<tr>
<td>10</td>
<td>191 ± 27.8</td>
<td>222 ± 21.4</td>
<td>219 ± 12.5</td>
</tr>
<tr>
<td>11</td>
<td>190 ± 6.26</td>
<td>218 ± 11.9</td>
<td>200 ± 35.4</td>
</tr>
<tr>
<td>12</td>
<td>191 ± 40.0</td>
<td>219 ± 37.4</td>
<td>194 ± 24.0</td>
</tr>
</tbody>
</table>

*Comparing day 12 with day 1 for each subgroup, p < 0.05.

Table 2. Pharmacokinetic parameters of halofantrine and desbutylhalofantrine in control and diabetic rats following oral administration of 8 mg/kg dose of halofantrine HCl.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Halofantrine (n = 4)</th>
<th>Desbutylhalofantrine (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>4.00 ± 0.82</td>
<td>4.75 ± 1.50</td>
</tr>
<tr>
<td>Cmax (ng mL⁻¹)</td>
<td>930.07 ± 145.96</td>
<td>406.40 ± 63.24*</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>52.57 ± 12.10</td>
<td>54.86 ± 14.15</td>
</tr>
<tr>
<td>AUC₀⁻⁻¹ (µg.hr mL⁻¹)</td>
<td>20.35 ± 1.15</td>
<td>18.76 ± 3.18</td>
</tr>
<tr>
<td>AUC₀⁻¹₂ (µg.hr mL⁻¹)</td>
<td>4.95 ± 0.14</td>
<td>2.91 ± 0.15*</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>65.68 ± 12.50</td>
<td>73.74 ± 14.39</td>
</tr>
<tr>
<td>Cmax/AUC₀⁻¹ (hr⁻¹)</td>
<td>0.046 ± 0.005</td>
<td>0.023 ± 0.003*</td>
</tr>
<tr>
<td>(AUC₀⁻¹₂/AUC₀⁻¹)₀⁻₇₂</td>
<td>0.42 ± 0.052</td>
<td>0.42 ± 0.068</td>
</tr>
</tbody>
</table>

*Significantly different at p < 0.05, when compared with the control.

significant increase on day 12 compared to day 1 for all the subgroups, with the values for glucose levels being as high as 2.5 - 5 times the control values (Table 1). The mean (± SD) plasma concentrations versus time profiles of HF and DHF in control and diabetic groups of rats following single oral administration of 8 mg/kg body weight doses of HF.HCl are depicted in Figures 1A and B while Table 2 shows the calculated pharmacokinetic parameters. The Cmax for HF and DHF in the control (930.07 ± 145.96 ng mL⁻¹ and 408.40 ± 42.80 ng mL⁻¹ respectively) were significantly higher (up to 2.5 times) than in diabetic rats (406.40 ± 63.24 ng mL⁻¹ and 188.68 ± 18.97 ng mL⁻¹ respectively). There was a marked decrease of up to 40% in the early AUC (AUC₀⁻¹₂) of HF and DHF while the AUC₀⁻¹ did not differ significantly (p > 0.05) in the diabetic compared to the control group. Also, the absorption rate calculated from the ratio, Cmax / AUC₀⁻¹, as given by Lacey et al. (1994), was up to 58% lower in the diabetic rats. The other pharmacokinetic parameters: Tmax, elimination half-life and Mean Residence Time of HF as well as the drug metabolic ratio obtained from the ratio of the metabolite AUC₀⁻₇₂ to that of the parent drug, were not significantly different (p > 0.05) between the two groups of rats (Table 2).

Table 3 shows the mean ± SD of plasma concentrations of total bilirubin, cholesterol, triglycerides and total protein in control and diabetic groups at indicated time points from day 12 after normal saline (for control) or alloxan administration. The mean plasma levels of bilirubin and cholesterol in the two groups showed no significant differences (p > 0.05). However, the mean triglycerides levels increased significantly while the total protein concentrations significantly decreased in the diabetic compared to the control (p < 0.05). The area under
Table 3. Mean plasma concentrations of total bilirubin, cholesterol, triglycerides and total protein in control and diabetic rats.

<table>
<thead>
<tr>
<th>Sub group</th>
<th>Mean plasma concentrations (Mean ± SD, n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Bilirubin (mg dl⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>0.75 ± 0.17</td>
</tr>
<tr>
<td>2</td>
<td>0.51 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>0.77 ± 0.24</td>
</tr>
<tr>
<td>4</td>
<td>0.59 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.56 ± 0.31</td>
</tr>
<tr>
<td>6</td>
<td>0.50 ± 0.27</td>
</tr>
<tr>
<td>7</td>
<td>0.64 ± 0.16</td>
</tr>
<tr>
<td>8</td>
<td>0.60 ± 0.22</td>
</tr>
<tr>
<td>9</td>
<td>0.40 ± 0.14</td>
</tr>
<tr>
<td>10</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>11</td>
<td>0.44 ± 0.24</td>
</tr>
<tr>
<td>12</td>
<td>0.56 ± 0.20</td>
</tr>
</tbody>
</table>

Mean±SD (n=12) 0.558±0.123 0.532±0.117 68.292±19.513 68.417±23.234 69.400±26.823 109.542*±46.220 378.167±142.590 228.000*±59.067

*Significantly different from control in each subgroup (n = 4) at p < 0.05.

The plasma concentration versus time curve (0 – 72 h) values for triglycerides increased by 46% while the total proteins decreased by 44% in diabetic compared to control rats. The ratios of the average plasma concentrations of HF, DHF, cholesterol, triglycerides and total plasma protein in the control versus diabetic groups at the different sampling time points showed that a good and significant positive linear correlation at p < 0.05 existed between the control/diabetic concentration ratios of HF and triglycerides (r > 0.66), and between the control/diabetic ratios of DHF and triglycerides (r > 0.62). The control/diabetic ratios of HF or DHF showed poor and non-significant linear correlation with cholesterol and total protein control/diabetic concentration ratios.

Results of in vitro binding studies of HF and DHF to plasma components showed that unbound fractions of halofantrine (HF) in control and diabetic groups were 0.104 ± 0.014 and 0.020 ± 0.008 (Mean ± SD), respectively, while the corresponding values for DHF were 0.147 ± 0.094 and 0.079 ± 0.022, respectively. Thus, the unbound fraction was significantly higher (p > 0.05) in control compared to diabetic. The increased drug binding in diabetes was more pronounced with HF which had a 5-fold decrease in the unbound fraction compared to a 2-fold decrease observed for its metabolite.

**DISCUSSION**

The decrease in weights and increase in blood glucose levels in the diabetic rats are consistent with the clinical signs and symptoms of diabetes mellitus. Day 12 post induction of diabetes was chosen to ensure a steady state blood glucose level, thereby allowing the use of the animals for pharmacological investigations.

The values of the pharmacokinetic parameters such as the T_max, C_max and AUC_{0-12} for the control rats were within the range of earlier report (Brocks and Toni, 1999). The significant reduction in the C_max of HF and DHF in the diabetic condition may have clinical implications since it has been asserted that the clinical efficacy of HF is influenced by its peak plasma concentration (Bryson and Goa, 1992). It was necessary to monitor the DHF plasma levels since the metabolite has anti-malarial activity which is equipotent to HF. The marked decrease in the early AUC (AUC_{0-12}) of HF and DHF in the diabetic compared to the
control group is suggestive that diabetes induced a reduction in rate of absorption of the drug. This assertion is further evident in the absorption rate calculated from the ratio, C_{max} / AUC_{0-\infty}, which was up to 58% lower in the diabetic rats. The plasma clearance and apparent volume of distribution of HF were not calculated and compared for the two groups as valid interpretations of any changes cannot be made since the effect of diabetes on oral bioavailability of the drug is not known. In the present study, the drug was administered only orally to the animals so as to reflect the therapeutic use of the drug whereby only oral dosage formulations are currently commercially available.

The role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs has been reviewed (Wasan and Cassidy, 1998). The significant increase in plasma triglycerides concentration and decreased plasma total protein concentration (Table 3) are consistent with the reported biochemical changes associated with diabetic condition (Nerurkar et al., 1983; Schonfeld et al., 1974). In order to ascertain the role of the three biochemical parameters in influencing the observed changes in HF disposition in diabetic rats, Pearson correlation analysis was performed to describe the relationship between plasma halofantrine or desbutylhalofantrine concentration ratio (control/diabetic) and the corresponding plasma cholesterol, triglycerides and total protein concentration ratios. A good and significant positive linear correlation (r > 0.60) (p < 0.05) observed between HF or DHF and the corresponding triglycerides ratios lend credence to the possible involvement of triglycerides in alteration of the pharmacokinetics of the drug in diabetes. Earlier reports have shown that HF distribution is primarily a function of the triglyceride lipid per lipoprotein fraction and, HF binds significantly to triglyceride rich lipoproteins (Humberstone et al., 1998; Wasan et al., 1999). HF, being more lipophilic than DHF, is expected to associate more with lipids than its metabolite and this is reflected in the in vitro binding studies which showed that the unbound fraction of HF is significantly lower than that of DHF in both diabetic and control groups. The 5-fold decrease in the unbound fraction (fu) of HF in the diabetic group relative to control, compared to about 2 fold increase in triglycerides levels (Table 3) suggests that other plasma components may be involved in the binding of the drug. Alpha-acid glycoprotein (AAG) levels which are known to increase in diabetes (Cenni et al., 1995; McMillan, 1989) can contribute to the increased HF plasma binding since the drug is a basic compound that can also bind to the glycoprotein, an acute-phase serum protein. Although there is no published report confirming that HF binds to alpha-acid glycoprotein, a review by Israili and Dayton (2001) points out that hundreds of drugs with diverse structures bind to the glycoprotein. Several plasma components are known to contribute simultaneously to plasma protein binding of drugs (Shibukawa et al., 2001). The rationale for measurement of total bilirubin in this study was not for assessment of its contribution to plasma drug binding but to serve as an indicator to rule out the possibility that alloxan administration might impair hepatic function that could affect the drug kinetics.

High drug concentrations applied in the in vitro binding studies were in conformity with the methods used by Brocks (2002) and this is due to the extensive binding of HF and DHF to plasma components. Binding of drugs to plasma proteins and lipoproteins plays an important role in the pharmacokinetics and pharmacodynamics of a drug as the free drug concentration, rather than the total concentration in plasma, correlates better with the drug effect (Kwong, 1985; Svensson et al., 1986; Levy and Moreland, 1984; Andreasen and Christiansen, 1990). But, the consequences of drug-lipoprotein binding differ from drug-protein binding as various lipoproteins receptors exist all over the body, and once bound, lipoproteins can be metabolized by the epithelial surface enzymes or
transported across cell membranes (Allison et al., 1994; Humberstone et al., 1998).

A drug such as HF that has a low hepatic extraction ratio (Brocks, 2000) and is highly bound to plasma proteins/components, a significant decrease in its unbound fraction (fu) would be expected to lead to a decrease in plasma Clearance, with a resultant increase in plasma drug levels. This has been demonstrated for HF where elevated plasma lipoprotein levels have been shown to result in significantly enhanced plasma drug concentrations due to reduced plasma clearance and volume of distribution of the drug (Humberstone et al., 1998). The present study shows contrary results as the increased plasma drug binding in diabetes was not associated with a significant change in the total drug exposure (AUC_{0-\infty}) compared to control values. This deviation from the expectation could be attributable to possible counter-balancing effects of other pharmacokinetic-modulating processes induced by diabetes. For example, apart from altered binding to plasma components, increased metabolism of the drug in diabetes is a possibility. The metabolism of HF is mediated mainly by CYP3A4 which is a homologous human form of CYP3A1(23) in the rat, and studies have shown that diabetes mellitus induces the enzyme activity (Halliday et al., 1995; Kim et al., 2005). Increase in the metabolism of clarithromycin, a CYP3A4 substrate, resulting in significant reduction in the plasma drug levels, has been reported for rats with experimental diabetes (Kim et al., 2005). Induced metabolism of HF in diabetes would have counterbalanced an increase in the plasma drug levels expected from the enhanced drug binding to plasma components. Although results of this study indicate that the metabolic ratios (AUC_{HF}/AUC_{HF}) of the drug are comparable between the two groups of rats, which apparently is suggestive of no evidence of induced metabolism of the drug, this might not be a reasonable index from which to discount the possibility of enzyme induction as counteracting the expected increased plasma HF levels in diabetes. Increased plasma drug binding in diabetes was more pronounced with HF which had a 5-fold decrease in the unbound fraction compared to a 2-fold decrease observed for its metabolite (DHF). Therefore, on the basis of the unbound fraction alone, the clearance of DHF relative to HF might be greater in diabetes thus blunting the higher AUC_{HF}/AUC_{HF} ratio expected with induced metabolism. This possibility is highly feasible because DHF has a pharmacokinetic profile similar to that of HF, each possessing low values of clearance and high volume of distribution (Brocks, 2000).

Another important factor that can affect the drug disposition in diabetes is disturbed gastrointestinal motility (Abrahamsson, 1995; Locke, 1995) which has been demonstrated to have a profound influence on the absorption of orally administered drugs (Richard and Murray, 1999). A significant decrease in gastric emptying rate has been reported in experimental diabetes (El-Salhy, 2002) which is similar to reported gastrointestinal hypomotility observed in patients with diabetes (Falwaczny et al., 1999). A slow gastric transit could explain the observed reduction in the rate of drug absorption in the diabetic compared to control group (Table 2). A decrease in the extent of the drug absorption might also contribute to counterbalancing the expected increase in the plasma drug levels in diabetes. Since Cmax is a function of both rate and extent of drug absorption, the significant reduction of this parameter for HF and its metabolite in diabetes supports the possibility of this explanation. A significantly decreased extent of GIT absorption of some drugs including furosemide has been observed in alloxan-induced diabetic rats (Park et al., 1998). This is in conformity with the report that the rate and extent of absorption of drug given orally could be expected to be altered in diabetes mellitus patients due to disorders of GIT (Guilt et al., 1991).

Changes in the glomerular filtration rate are known to be induced by diabetes (Mogensen, 1971; Jensen et al., 1981). Since HF undergoes insignificant renal excretion with less than 1% of the administered dose excreted as unchanged drug in urine (Brocks and Toni, 1999), an alteration in glomerular filtration rate is not expected to have any effect on the disposition of the drug in diabetes. From the present study, therefore, elevated plasma drug levels expected in diabetes due to the observed marked increase in drug binding to plasma components appears to be counterbalanced by other processes. Further studies are required to characterize the degree of contributions of induction of metabolism and/or reduction in GIT drug absorption in alteration of pharmacokinetics of HF in diabetes.

In conclusion, this study shows that diabetes induces an alteration in the pharmacokinetics of halofantrine and its major metabolite, especially the C_{max} drug absorption parameters and binding to plasma components. Elevated plasma drug levels expected in diabetes due to observed marked increase in drug binding to plasma components appears to be counterbalanced by other pharmacokinetic-modulating processes. The significantly reduced C_{max} of the drug and its metabolite in diabetes may have clinical implications since the clinical efficacy of HF is influenced by its peak plasma concentrations. Further studies are required to confirm these pharmacokinetic changes in diabetic humans.

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