Influence of honey on orally and intravenously administered diltiazem kinetics in rabbits

K Koumaravelou, C Adithan*, C H Shashindran, Azad Mohammed & Benny K Abraham

Department of Pharmacology, Jawaharlal Institute of Post Graduate Medical Education and Research, Pondicherry 605 006, India

Received 15 March 2002; revised 2 August 2002

Effect of honey on plasma concentration of diltiazem after oral and intravenous administration in rabbits, has been studied. For oral study, single dose of diltiazem (5mg/kg. po) along with saline was administered to New Zealand white rabbits (n=8). Blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 and 8 hr after drug administration from marginal ear vein. After a washout period of one week, diltiazem was administered with honey (2.34ml/kg; po) and the blood samples were collected as above. To the same animals honey (2.34ml/kg; po) was continued once daily for 7 days. On 8th day, honey and diltiazem were administered simultaneously and blood samples were collected at similar time intervals as mentioned above. For intravenous study the pharmacokinetic was done in each animal on two occasions. The first study was done after single dose administration of diltiazem (5mg/kg; iv) along with saline (2.34ml/kg; po). Blood samples were collected at 0, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 and 6 hr after iv diltiazem administration. The same animals were treated with honey (2.34 ml/kg; po) for seven days. On day 8, the second study was carried out with single dose iv administration of diltiazem along with honey (2.34ml/kg; po) and blood samples were collected. In the oral study, single dose administration of honey decreased the AUC and Cmax of diltiazem associated with significant increase in clearance and volume of distribution when compared to saline treated group. After one week administration of honey, diltiazem kinetic data showed further reduction in AUC and Cmax and increase in clearance and volume of distribution. In the iv study also, multiple dose administration of honey significantly reduced the AUC and increased the clearance value of diltiazem. The results suggest that honey may decrease the plasma concentration of diltiazem after its oral or iv administration in rabbits.

Food-drug interaction can be associated with alterations in the pharmacokinetic and pharmacodynamic profile of various drugs that may have clinical implications\(^1\).

Diltiazem hydrochloride is a calcium channel blocker used in the treatment of arrhythmia, angina pectoris and hypertension\(^2\). It undergoes variable and extensive first pass metabolism by CYP3A present in intestinal wall and liver before entering into systemic circulation\(^3\,4\).

From olden days, honey is used to mask the bitterness of the drugs, advocated as a rapid source of energy and also as a panacea for various illnesses\(^5\). Honey is a saccharine substance made by the hive bee, *Apis mellifera* Linn. (order Hymenoptera, Family Apidae), and other species of *Apis*, from nectar of flower\(^6\). Honey consists of about 70 to 80 % mixture of glucose, fructose, small amount of sucrose (1.1 to 4.4 %) and dextrin (0.06 to 1.24 %) and trace elements like hydrogen peroxide, lysozymes\(^7\) and other organic substances. A small amount of volatile oil, wax and pollen grains are also usually present\(^8\).

Although humans commonly consume honey, its effect on drug metabolism in humans is not studied. In rabbits, we have reported earlier that the carbamazepine plasma concentration was decreased by the concomitant administration of honey\(^9\). Carbamazepine and diltiazem are metabolized by CYP3A enzyme. In the present study, the effect of honey on diltiazem pharmacokinetic, after oral and iv administration, was investigated in the rabbits.

**Materials and Methods**

**Chemicals**—Pure reference standard, tablets and injection of diltiazem were obtained from Torrent Pharmaceuticals Ltd. (India). HPLC grade acetonitrile and triethanolamine were from S.D Fine Chemicals (India) and Spectrochem Pvt Ltd. (India) respectively. Analytical grade dibasic sodium phosphate, and orthophosphoric acid were from S.D. Fine Chem Ltd (India). Double distilled water was used.

Honey was obtained from Periyakulam Sarvodaya Sangh, Periyakulam, India, and was tested for its purity in Public Health Laboratory, Pondicherry, India. It was within PFA (Prevention of food adulteration act-1955, India) values (moisture 24%, ash 0.3%, sucrose 2.4%, reducing sugar 71.6%, fructose/glucose ratio 0.97).

\(^{*}\)Correspondent author:
E-mail : adithan@vsnl.com
**Animals**—New Zealand white rabbits (n=8, 2 to 2.5 kg), obtained from Hessargatha Livestock Station, Bangalore, India, were used in this study. They were housed in individual cages and had free access to food and water.

**Experimental Design**

I. Oral study of diltiazem: The pharmacokinetic evaluation of orally administered diltiazem was done on each animal on three occasions. The first study was done after single dose administration of diltiazem (5mg/kg; po) along with saline (2.34ml/kg; po). After a washout period of one week, the second study was conducted with single dose administration of diltiazem and honey (2.34ml/kg; po). After this, the animals continued to receive honey (2.34ml/kg; po) once daily for 7 days. On 8th day the third pharmacokinetic study was carried out after oral dosage of diltiazem and honey.

II. Intravenous study of diltiazem: The pharmacokinetic evaluation of iv administrated diltiazem was done on each animal on two occasions.

The first study was done after single dose administration of diltiazem (5mg/kg; iv) along with saline (2.34ml/kg; po). The animals were treated with honey (2.34ml/kg; po) for seven days. On day 8 the second study was carried out with single dose administration of diltiazem along with honey (2.34ml/kg; po).

During each pharmacokinetic study, the animals were fasted overnight and blood samples were collected (approximately 1ml) into screw capped eppendorff tube from a heparinized 22 G iv cannula (Mediflon, India) inserted into the marginal ear vein. A separate iv cannula was inserted into the marginal ear vein for iv administration of diltiazem.

**Sample collection and storage**

The blood sampling times were 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 and 8 hr after oral administration of diltiazem and 0, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 and 6 hr after iv diltiazem administration.

Each sample was centrifuged and the plasma was separated and stored at -20°C until analysis so as to prevent metabolic changes.

**Diltiazem assay**

The plasma diltiazem level was estimated by HPLC method of Chaudhary et al.\(^\text{12}\), with a slight modification using verapamil as internal standard. An ODS column was used and the mobile phase consisting of 40% acetonitrile in 0.01 M dibasic sodium phosphate and 0.1% (v/v) triethanolamine dissolved in water (double distilled). The pH of the mobile phase was adjusted to 3.0 ± 0.1 with 85% (w/v) orthophosphoric acid. The flow rate was 1.5ml/min. Sample detection was performed with an UV-detector (SPD-6AV-Shimadzu, Japan) at a wavelength of 237 nm and 0.1 absorbance units full scale (a.u.f.s). Extraction of diltiazem from plasma was done by the method described by Rustum et al.\(^\text{13}\). The assay limit of detection was 1 ng/ml. The inter day and intra day co-efficient of variation (CV) at the concentration 1.5, 10 and 20 ng/ml were all less than 5% (n=10).

**Pharmacokinetic analysis**

**Oral administration**—The pharmacokinetic analysis was carried out using model independent formulae. The log concentration of the drug in plasma was plotted versus time for each animal.

The apparent elimination half life (t\(_{1/2\text{p}}\)) of the drug was obtained from the least-squares linear regression of the terminal portion on the log concentration vs. time plot. The area under the drug concentration-time curve from 0 to time of the last measured concentration (AUC\(_{\text{t-o}}\)) was calculated using trapezoidal rule. The extrapolated area under the curve (AUC\(_{\text{t-o}}\)) was calculated as C/K\(_{\text{el}}\), in which C is the last measured concentration, and K\(_{\text{el}}\) is the terminal elimination rate constant and added to AUC\(_{\text{t-o}}\) to obtain area under the curve from 0 to infinity (AUC\(_{\text{t-o}}\)). The serum peak concentration (C\(_{\text{max}}\)) of diltiazem was read directly from the actual plasma data of 8 rabbits.

The total plasma clearance (CL) was calculated as F* Dose/AUC\(_{\text{t-o}}\), where F is the bioavailability. F was calculated by dividing AUC\(_{\text{t-o}}\) after oral administration by the AUC\(_{\text{t-o}}\) after iv administration.

The total volume of distribution (Vd) was calculated using the formula F* Dose/ (AUC\(_{\text{t-o}}\)*K\(_{\text{el}}\)).

The absorption rate constant K\(_{\text{a}}\) was calculated by Wagner Nelson method\(^\text{14}\) using Topfit Pharmacokinetic software.

**Intravenous administration**—The area under the drug concentration curve and elimination half life were calculated using the same method followed for oral study.

Clearance rate of the drug (CL) was calculated by Dose/AUC. The volume of distribution (Vd) was by Dose/AUC*K\(_{\text{el}}\). C\(_{0}\) was calculated by extrapolating the terminal linear portion of plasma drug concentration curve to time zero.
Statistical analysis

The relationship between the pharmacokinetic parameters of the studies after oral administration was examined by Turkey Multiple comparison test. Paired Student’s 't' test was used to check the level of significance for the data after intravenous drug administration. $P < 0.05$ was considered to be indicative of significance.

Results

Effect of honey on orally administered diltiazem

Plasma diltiazem concentration after co-administration with single dose of honey was found to be decreased in comparison with its concentration when co-administered with saline (Fig. 1). A significant reduction was observed in maximum peak plasma concentration ($C_{max}, P < 0.01$) and AUC ($P < 0.01$) of diltiazem after single dose honey treatment when compared to saline control. CL and Vd of diltiazem showed a significant ($P < 0.01$ and $P < 0.05$ respectively) increase when compared with saline control (Table 1). The $t_{1/2}$ was not significantly affected by single dose honey treatment.

Multiple dose honey treatment showed a further reduction in the AUC ($P < 0.01$) and $C_{max}$ ($P < 0.01$) of diltiazem associated with significant increase in the clearance rate ($P < 0.01$) and Vd ($P < 0.05$) when compared with saline control (Table 1). No significant change in other pharmacokinetic parameters was observed.

Effect of honey on intravenously administered diltiazem

After seven days of honey treatment, the plasma concentration of diltiazem showed a significant decrease when compared with saline treated group (Fig. 2). It also significantly reduced the AUC ($P < 0.05$) and $C_{max}$ ($P < 0.05$) when compared with saline treated group. A significant increase in clearance ($P < 0.05$) was observed. There was a nonsignificant increase in the volume of distribution (18%) was observed when compared with saline treatment.

![Graph 1](image1.png)

**Fig. 1** — Effect of single and multiple dose of honey (Hn) on the mean plasma concentration of orally administered diltiazem (DTZ). Values are mean ± SE, N=8. *$P < 0.01$; **$P < 0.05$.

![Graph 2](image2.png)

**Fig. 2** — Effect of multiple dose of honey (Hn) on the mean plasma concentration of intravenously administered diltiazem (DTZ). Values are mean ± SE, N=6. *$P < 0.02$; **$P < 0.01$.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DTZ Alone</th>
<th>DTZ + single dose honey</th>
<th>DTZ + multiple dose honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/ml/h)</td>
<td>165.26 ± 13.84</td>
<td>97.085 ± 13.68**</td>
<td>66.47 ± 15.42**</td>
</tr>
<tr>
<td>CL (L/min/Kg)</td>
<td>0.37 ± 0.03</td>
<td>0.68 ± 0.09</td>
<td><strong>2.47 ± 1.135</strong></td>
</tr>
<tr>
<td>Vd (L/Kg)</td>
<td>0.07 ± 0.007</td>
<td>0.12 ± 0.02</td>
<td><strong>0.49 ± 0.27</strong></td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>2.13 ± 0.28</td>
<td>2.17 ± 0.34</td>
<td>3.88 ± 1.22</td>
</tr>
<tr>
<td>$K_{e}$ (hr⁻¹)</td>
<td>0.76 ± 0.000</td>
<td>0.54 ± 0.33</td>
<td>0.83 ± 0.16</td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>71.42 ± 4.713</td>
<td>33.90 ± 5.29**</td>
<td>15.52 ± 4.972**</td>
</tr>
</tbody>
</table>

*$P < 0.05$, **$P < 0.01$ when compared to diltiazem alone.
The reduction in the bioavailability of diltiazem could also be due to the decrease in the oral absorption. It has been reported that elevation of the viscosity of the contents in the upper GI tract can reduce drug absorption. The presence of a viscous chyme generated by a solid meal can act as a physical barrier, thereby reducing drug access to the absorptive surface of the GI tract. Hence, the decrease in the plasma concentration of diltiazem could also be due to the viscosity of honey.

In both oral and iv study, there was a significant increase in the clearance of diltiazem but there was no significant change in the t1/2. This is due to the fact that in parallel with the increase in CL, the Vd of the drug also increased. Since t1/2 is a parameter derived (i.e., t1/2 = 0.693 X Vd/CL) from CL and Vd, there is no significant change in the elimination half-life of the drug. The increase in the CL may be due to the induction of CYP3A enzyme. However the reason for increase in the Vd is not known which needs further investigation.

In order to rule out the possibility that repeated blood withdrawal from the same animal (2 kinetic study within 9 days period) may be responsible for the decrease in the bioavailability of diltiazem, a pilot study was carried out by giving diltiazem intravenously along with oral saline. There was no difference in diltiazem kinetics (data not shown). Hence the reduction in bioavailability may not be due to haemodynamic changes subsequent to repeated withdrawal of blood.

To conclude, honey decreases the plasma concentration of orally and intravenously administered diltiazem, which may be due to induction of CYP3A enzyme in rabbits. This data needs to be confirmed by human study.

Acknowledgement

The authors thank Dr. Srikumaran K. Melethil (Professor, Department of Pharmaceutics and Medicine, School of Pharmacy, University of Missouri - Kansas City) for his valuable suggestions and support during the course of the study.

Table 2—Effect of honey on pharmacokinetic parameters of intravenously administered diltiazem (DTZ) [Values are mean ± SE. N= 6]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DTZ Alone</th>
<th>DTZ+ multiple dose honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/ml/h)</td>
<td>226 ± 12.3</td>
<td>144 ± 10.95*</td>
</tr>
<tr>
<td>CL (L/min/Kg)</td>
<td>0.42 ± 0.04</td>
<td>0.59 ± 0.04*</td>
</tr>
<tr>
<td>Vd (L/Kg)</td>
<td>0.05 ± 0.005</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.63 ± 0.15</td>
<td>1.64 ± 0.38</td>
</tr>
<tr>
<td>Kel (h^-1)</td>
<td>0.44 ± 0.04</td>
<td>0.43 ± 0.08</td>
</tr>
<tr>
<td>C0 (ng/ml)</td>
<td>118.00 ± 2.65</td>
<td>106.8 ± 5.07*</td>
</tr>
</tbody>
</table>

*P < 0.05 when compared to diltiazem alone.
City, Kansas City, Missouri, USA) for his valuable suggestion regarding the work and Mr. Balakrishnan, R, Mrs. Tamijarasdy, Mrs. Immaculate for their technical assistance.

References