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Research Article

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Acidic beverage and the bioavailability of theophylline

Abdurrahim A. Elouzi[1*], Fadel Abeid[2], Mohamed Almegrhe[3] and Mokhtar El-Baseir[1]

[1]Department of Pharmaceutics, Faculty of Pharmacy, University of Tripoli, Tripoli-Libya.
[2]Tripoli Central Hospital, Tripoli-Libya.
[3]Biotechnology Research Center, Twisha, Gasr ben Ghachier, Tripoli-Libya

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ABSTRACT

In this study the effect of acidic beverages (Pepsi) on the bioavailability of theophylline was investigated in healthy rabbits. Assuming an open one compartment model, theophylline suspension (100mg/kg) was administered orally via intragastric tube. Blood samples were withdrawn at time intervals from 0-24hr. after washing period of 7 days, Pepsi (10ml/kg) was administered concomitantly with theophylline (100mg/kg) and then blood samples were taken at similar intervals. Plasma was separated and treated in a specific manner and assayed for theophylline by using high performance liquid chromatography (HPLC). The data calibration curve was constructed and various pharmacokinetic parameters were calculated. Comparisons of data revealed significant differences between the pharmacokinetic parameters for theophylline given alone and with pepsi. The results suggest a significant increase in the bioavailability of theophylline in presence of pepsi that ultimately will lead to the appearance of unwanted side effect and toxic response.

Key words: Theophylline, Pepsi, rabbits, bioavailability, beverages

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INTRODUCTION

Theophylline with the structure as in Figure 1 is a potent bronchodilator, with a narrow therapeutic index in serum concentration for the therapeutic range of 5–20 mg/L [1]. Theophylline occurs as a white, odorless crystalline powder with a bitter taste, structurally is classified as dimethylxanthine, used in therapy for chronic obstructive pulmonary disease under a variety of brand names. It is found in cocoa beans, as high as 3.7 mg/g [2]. The first clinical use of theophylline was in 1902 as diuretic, 20 years later described in asthma treatment [3]. Although, the bioavailability of theophylline is almost 90-100 %, taking the drug late in the evening may slow the absorption process, without affecting the bioavailability. Theophylline is metabolized extensively in the liver (up to 70%) by parallel first order and Michaelis-Menten pathways. Metabolism may become saturated even within the therapeutic range. A number of factors have been identified that make it difficult to predict the dose needed to achieve a desired plasma level in patients. These factors include variations due to absorption and distribution. Any interaction may result in disproportionately large increases in serum concentration of theophylline can lead to unwanted toxic effect, whereas a decrease in bioavailability leads to loss of therapeutic effect [4]. Theophylline is excreted unchanged in the urine (up to 10%). Clearance of the drug is increased in children 1 to 12, teenagers 12 to 16, adult smokers, elderly smokers, cystic fibrosis, and hyperthyroidism. It is known that the acidic drugs are rapidly absorbed from the stomach in unionized form and distributed to the blood circulation [5]. Pepsi is an acidic carbonated beverage consuming throughout the word. Other acidic beverages such as Coca-Coal have been reported to increase the absorption of some drugs such as ketoconzole [6] and itraconzole [7]. In theory, if more acidic gastric media for the drug is provided by the administration of an acidic beverage (Pepsi) the unionized portion of the drug in the stomach will be increased and diffusion through the stomach mucosal will be easier leading to a high serum concentration of the drug [5]. Pepsi contains caffeine which is known to work in similar way to theophylline. Since the effect of both

components didn't differ significantly, therefore caffeine can reduce the elimination of theophylline. So taking theophylline with beverage containing caffeine (Pepsi) may increase its effect. The aim of this study was to correlate the bioavailability of theophylline in presence of an acidic beverage on rabbits.

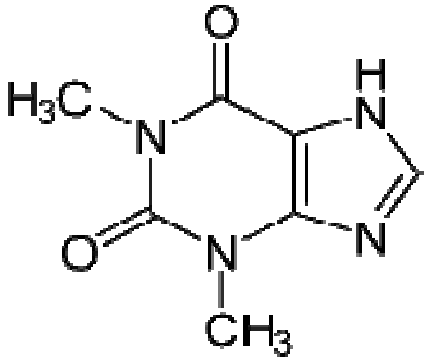

Fig 1. Chemical structure of Anhydrous Theophylline.

EXPERIMENTAL SECTION

Materials

Theophylline powder from Sigma was a gift from Strathclyde Institute for Medical Science. Bronchophylline tablets from Dar Essaysali Sfax (Tunisia) were bought from local pharmacy. Lignocaine was from the Arab cairo ARE. Teething gel (Dentinox) was from England-UK, Milli-Q plus water was from Millipore, Bedford, MA, U.S.A. The solvents used were of analytical or HPLC grade.

Animal experiment

Eight female Albino New Zealand white rabbits, weighing 1.9-2.2 Kg were used throughout the study**.** Animals were housed in pairs in stainless steel cages with constant temperature environment (23 -33°C) and 12 hours of light each day prior and through the study. Animals were provided with chlorinated tap water through a lixit at all times and food by a J-hopper attached to the front of the cage. Cage cards were utilized to identify and to avoid misidentification of the animals and a temporary identification by pen marks on the fur and ear of individual rabbit were used. Animals were received a high fiber diet in a specified amount to minimize common gastrointestinal disorders and to prevent obesity. To prevent the development of allergies and to provide some safety from rabbit scratches the latex gloves were wear through all the study. The experiment was initiated after three months of training on how to handle the animal and withdrew the blood from the marginal vein**.**

Experimental design:

In this study an open, cross over design was adopted to investigate the effect of acidic beverage (Pepsi) on the bioavailability of theophylline. Animals were weighed and the doses of sedating agent (combistress) and theophylline were calculated. Theophylline suspension (100mg/kg) of animal weight was prepared by grinding 28 theophylline tablet using clean dry mortar and pestle and then the calculated dose was dissolved/suspended in 9 ml distilled water. Animal was given subcutaneously sedating dose and when it starts to show signs of sedation, the dose of theophylline suspension (100mg/kg) was given orally through intra-gastric tube covered with Dentinox® gel to facilitate the entrance into the stomach and after spraying the mouth of the animal with local anesthetic (Lignocaine). Blood samples (1ml) were drawn via ear vein at 0, 0.5, 1, 2, 4, 8, 16 and 24 hr of administration of theophylline. After a wash out period of 7 days, rabbits were orally given pepsi (10ml/kg) concomitantly with theophylline (100mg/kg) and then blood samples were taken at similar intervals and left to stand for 0.5 hour prior to centrifugation at 3000 rpm for 7 min. The plasma was separated and pipetted into 2 ml plastic tubes and kept frozen at -20 °C until analysis for theophylline by reverse phase high performance liquid chromatography (HPLC) [8].

Analytical method

High Performance Liquid Chromatography (HPLC), Shimadzu Corporation, Kyoto, Japan. was used to quantitatively detect theophylline in the plasma. The HPLC apparatus included one pump (LC-6AD, Shimadzu, Japan) and a chromatopac (C-R6A, Shimadzu, Japan). The assay employed an Inertsil C_{18} column (25mm×46mm, 5 µm). Chromatographic separation was achieved by using a mobile phase consisting of acetonitrile and water (25:75v/v) with a flow rate of 1.0 ml/min. An UV detector was set at 275 nm.

Plasma samples

Eppendorf tubes contained the plasma were thawed. Aliquots of 200µl of plasma sample transferred to a new eppendorf tube, acetonitrile (400µl) was added, vortexed for 1 min prior to centrifugation at 3000 rpm for 3 min. The clear supernatant filtered through 0.25 µm pore size filter into another microtube and evaporated to dryness by blowing of nitrogen. The residue was reconstituted with 100 µl of the mobile phase and 20 µl of the solution was then injected into HPLC system.

Standard calibration curve

A stock solution (2 mg/ml) of anhydrous theophylline in water was prepared. From the stock solution concentrations of 0.25-20 µg/ml were prepared. 200µL theophylline solution was spiked into 200µL plasma, vortexed for 1min, and then centrifuged at 3000 rpm for 3 min. 20 µL of the solution subjected to HPLC analysis as described above to establish standard curves. The calibration curves were drawn after linear regression of the peak-area ratios with correction of theophylline.

pH of beverage

To confirm that the beverages have got acidic media, the pH of most popular beverages use was measured using a digital pH meter (Thermo Fisher Scientific Inc.). The potentiometer was calibrated at pH 4.0 and 7.0 using standard buffer solutions. The pH of each solution was measured immediately after the canned beverage was opened.

Pharmacokinetic parameters Calculations

The following pharmacokinetic parameters of theophylline were estimated after oral administration assuming an open one compartment model; the total area under the plasma concentration-time curve from time 0 to time infinity (AUC) [9] was calculated using the trapezoidal rule-extrapolation method [10-12]. The peak plasma concentration (C_{max}); the time of Cmax, occurrence (T_{max}) were directly obtained from the plasma concentration-profiles. The absorption half life ($t_{½a}$) was calculated by the residual method; the elimination half life ($t_{½el}$) and the elimination rate constant Ke were calculated by least square regression analysis of the data points in the elimination phase of the semilogarthmic plot of theophylline plasma concentration versus time curve.

Data Statistical analysis

Statistical analysis was carried out on the data using analysis of variance followed by one-way analysis of variance (ANOVA) in trends of possible clinical importance and post tests carried out using Fisher's pair wise comparisons with the statistical package Minitab ^{TM13} windows. ***P*** value of less than 0.05 was considered to represent a statistically significant difference. Data are reported as means ± standard deviations.

RESULTS

The HPLC chromatogram of theophylline in a serum sample is shown in Fig 2. The linearity of calibration curve Y = 46048 X (R^2 = 0.961) was found within the range 0.25～5.0 µg/ml.

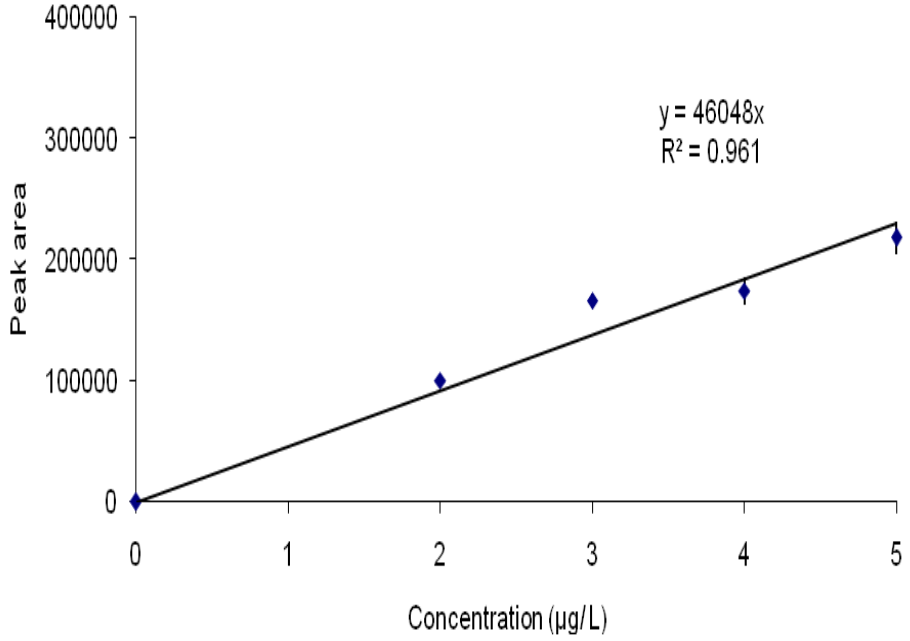

Fig 2. Calibration curve for serum theophylline concentration. Each point represents the mean of triplicate analysis.

The mean plasma concentration–time curves of theophylline before and after single dose of pepsi in rabbits are shown in Fig 3.

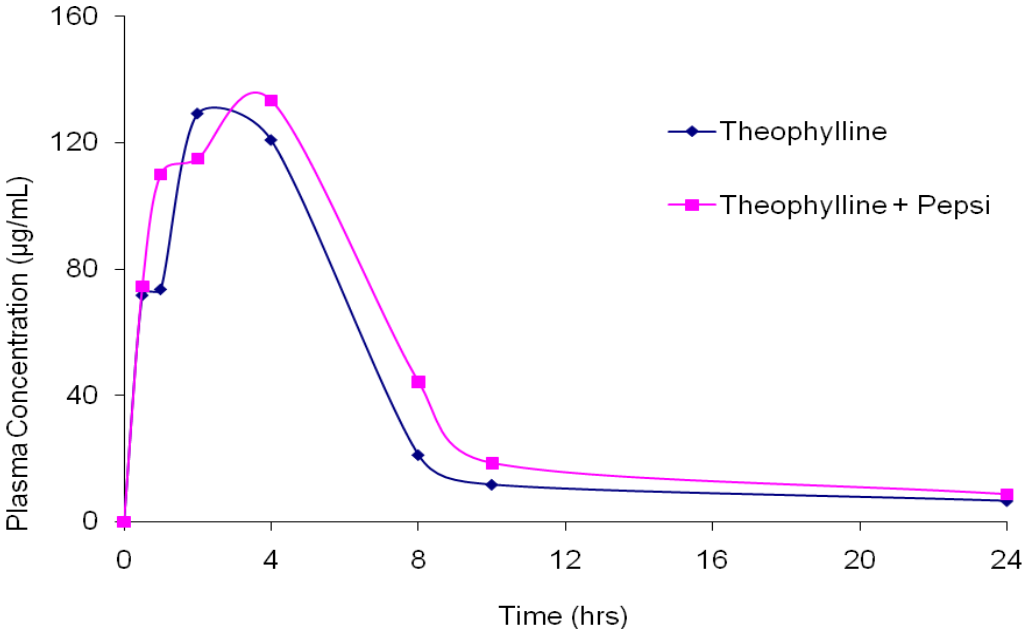

Fig 3. Plasma concentration of theophylline versus time in absence and after single dose of Pepsi.

The plasma theophylline levels were significantly increased at 0.5 to 10h after single dose of pepsi, compared with theophylline alone. Double peaks in the serum concentration were noted following administration of theophylline suspension. The first peak occurs at approximately (1.5 h) the time expected for the conventional tablet while the second peak at (2.4 h) is the expected time for colonic absorption. The trough between the two peaks is probably due to poor absorption and thought to be in caecum where the pH is low. The mean ± SEM of various pharmacokinetic parameters (Cmax, Tmax, $t_{1/2}$, $AUC_{0–24}$, $AUC_{0–\infty}$ and K_{el}) of theophylline before and after single dose of pepsi in rabbits are summarized in Table 1.

Table 1. Pharmacokinetic parameters of oral theophylline alone and after giving theophylline along with pepsi in eight rabbits.

Parameters	Theophylline alone	Theophylline + Pepsi
AUC_{0-24} (µg/ml.• h)	128 ± 11	190± 10
$AUC_{0-\infty}$ (µg/ml.• h)	142.19	210.09
C_{max} (µg/ml)	129.2 ± 10.3	133.44 ± 19.27
T_{max} (h)	2	4
K_{el} (h-1)	0.46	0.43
$t_{1/2\,el}$(h)	1.5	1.61
$t_{1/2\,a}$(h)	0.5	1

Results are given as mean ±S.E. (n = 8)
p < 0.05

A significant difference between the calculated pharmacokinetics parameters of theophylline given alone and after dose of Pepsi was observed. The results indicate a significant increase in the extent of absorption ($AUC_{0-24,}$ $AUC_{0-\infty}$, C_{max},) of theophylline given after pepsi compared to theophylline alone. The administration of pepsi also significantly ($p < 0.05$) prolonged the T_{max} from 2 h to 4 h and the $t_{1/2a}$ from 0.5 h to 1h respectively. These changes would probably explained on the bases that the acidic pH of pepsi (Table 2) lead to an increase in the acidity of the stomach and consequently an enhancement of theophylline absorption.

Table 2: pH values of the most commonly beverages used

Type of beverage	pH
Pepsi	2.5
Coca-Cola	2.5
Seven up	3.3
Fanta	2.87
Miranda	3.10
Diet Pepsi	3.3

Furthermore, presence of caffeine in pepsi which is known to work in similar way to theophylline [13]. No statistically significant difference was observed in the elimination half life ($t_{1/2el}$) and the elimination rate constant (Ke) of theophylline before and after single dose of pepsi.

DISCUSSION

Theophylline has maintained a crucial role as an effective and useful bronchodilator. Nevertheless the use of theophylline is often restricted by its narrow therapeutic range as a consequence the monitoring of plasma concentration in patients on theophylline treatment is now current practice. The management of the theophylline in clinical practice is complicated by several factors which influence the disposition of the drug, e.g., disease, obesity, age, smoking habits, diet, and drugs all contribute to the wide inter-individual variability of theophylline clearance. Measurement of plasma concentration of this drug is useful in evaluated patient compliance with prescribed dosage and in attaining therapeutic concentration while avoiding symptoms of unwanted side effect. The results of the present study show a significant increase in the extent of absorption (($AUC_{0\text{-}24,}$ $AUC_{0\text{-}\infty}$, C_{max},) of theophylline after single dose administration of acidic beverage (pepsi) compared with the control group. It is reported that the food and nutrition are considered to be important factors both in the absorption and the metabolism of drug. Depending upon the type of food, and degree of interaction, drug absorption might be reduced, delayed, unaffected or increased [14, 15]. A small alteration in the bioavailability would be unimportant for a drug with a wide therapeutic index however may be vital for a drug with sharp therapeutic range [15]. The pharmacokinetics of a single dose of theophylline was reported to be effected when it is given along with grapefruit juice [16]. Theophylline is a weak acid with pKa of 8.8. It has inadequate aqueous solubility in water but has the ability to solubilize in an acidic medium [17] which may be a possible explanation for the increase in the $AUC_{0\text{-}24,}$ $AUC_{0\text{-}\infty}$, and C_{max}, of theophylline after single dose of pepsi. It is demonstrated that the oral drug bioavailability can be markedly influenced by physiological factors, such as gastrointestinal pH, gastric emptying, small intestinal transit time, bile salt, and absorption mechanism [18]. Thus by understanding the physicochemical properties of a compound and also by recognizing the physiological process affecting drug absorption, might be helpful in better management of patient care.

CONCLUSION

The present study have shown that the administration of acidic beverage containing caffeine along with theophylline could increase the bioavailability of the drug consequently may lead to an increased incidence of the unwanted side effect and the toxic response.

Recommendation

This recent study promotes a further investigation to be done in healthy human volunteers and patient, if similar results to those shown here are obtained, clinician should either adjust the needed dose or advise their patient who are receiving theophylline to avoid consumption of acidic beverage such as pepsi to avoid the undesired effect.

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