Comparative Study of the Effects of Enzyme Inhibitors and Inducers on Serum and Tissue Availability of Thiamine After Single Oral Dose of the Pro-drug Benfotiamine in Rats.

Mustafa Ghazi Alabbassi* Munaf Hashim Zalzala and Saad Abdulrahman Hassain***

الخلاصه :

من المعروف أن للثيامين دور مهم في حماية أنواع مختلفة من ألخلايا والأنسجة من التاثيرات السمية للادوية والسموم. ان من أهم المشا كل التي تحدد التطبيقات السريرية في هذا المجال هو قلة أ متصاص الثيامين وتوافره الحيوي . تم حل هذه المشكله بأستخدام البنفوتيامين ذو القابليه على الذوبان في الدهون. لقد تم تصميم هذه الدراسه لتحديد مستوى الثياميين في مصل الدم والانسجه عند الجرذان بعد أعطاء جرعه واحده عن طريق الفم من ماده البنفوتيامين ومقارنتها مع جرعه مماثله من ماده الثيامين . بالاضافه الى ذلك دراسه تأثيرات محفزات ومثبطات الأنزيمات بأستخدام تقنيه التحليل الكروموتوغرافي السائل عالي الكفائة(HPLC). بناء" على ماتم التوصل أليه من نتائج يمكن الأستنتاج بأن البنفوتايمين أكثر توافرا حيويا في مصل الدم والكلى ماعدا الدماغ الذي يحتاج ألى وقت أطول.

Abstract

Thiamine is known to have an important role in the protection of different types of cells and tissues against the damage produced by many drugs and toxins. The most important problem limiting the clinical applications of this approach is the poor absorption and bioavailability of thiamine from the sites of administration, a problem which can be solved by the use of the lipid soluble pro-drug for thiamine, benfotiamine. Accordingly, this project was designed to evaluate the serum and tissues availability of thiamine in rats after the administration of single oral dose of benfotiamine compared with that produced by the same oral dose of thiamine, in addition to study the effects of enzyme inducers and inhibitors in this respect utilizing HPLC technique. According to the results obtained in this study one can conclude that thiamine availability after administration of benfotiamine was more in serum, liver, kidney while in the brain more time may be required to reach maximum level.

Introduction

Benfotiamine (S-benzyolthiamine-O-monophosphate) is a synthetic derivative of thiamin, belonging to the family of compounds known as "allithiamines." Benfotiamine is fat-soluble and thus more bioavailable and physiologically active than thiamin. Characteristic of the allithiamines is an open thiazole ring within the chemical structure of these thiamine-related compounds, making them lipid soluble. In contrast, thiamine, which is water soluble, has a closed thiazole ring. The lipid solubility of benfotiamine, conferred by this open ring, increases its bioavailability and it is readily absorbed at

***Department of Pharmacotherapy, College of Pharmacy, Almustansyria University, Baghdad Iraq.**

****Department of Pharmacology and Toxicology, College of Pharmacy, Baghdad University, Baghdad Iraq**

higher doses, in contrast to absorption of water-soluble thiamin salts, which decreases at higher doses, due to saturation of absorption sites in the intestine(1). The problem that faces the use of thiamine is the limited activity of the biological system to absorb and distribute thiamine effectively. Thiamine can not pass directly across the cell membranes since it require a special transporting system to pump it actively across intestinal wall and later into cellular compartments (2). Thiamine crosses intestinal wall through diffusion process to the blood stream and then to the extra cellular fluid; the cells themselves (except for red blood cells) can not uptake conventional thiamine easily, except through active transport systems (3). Benfotiamine readily passes through intestinal mucosal cells, where it is converted into physiologically active thiamine (4). Benfotiamine increases blood levels of thiamine pyrophosphate (TPP), the biologically active thiamin co-enzyme (5).It is also found that lipid-soluble prodrug benfotiamine resist destruction by the enzyme thiaminase, since it is converted to the biologically active form thiamine pyrophosphate(TPP)which is involved in the carbohydrate metabolism(6). Thiamine and its Co-enzyme, TPP Thiamine (vitamin B1) plays an essential part in the metabolism of glucose, through actions of its co-enzyme TPP (thiamine pyrophosphate) (7).

In this study we used phenobarbitone as enzyme inducer and cimetidine as enzyme inhibitor, since these two drugs act mainly on cytochrome P450enzyme family, in order to see if these two drugs affect the availability of thiamine in serum and tissues. Drug interactions involving the cytochrome P450 system are common, and generally result from either enzyme inhibition or induction. The effects of enzyme induction are sometimes more difficult to predict because these are dependent on drug half-lives, the rate of enzyme production, and individual genetic variations (8, 9).

Materials and Methods

This study was carried on twenty-five male adult rats during the period of August 30, 2005 to October10, 2005.The animals were housed in the animal house of Drug research and Quality Control Center/Ministry of Health under condition of controlled temperature, allowed free access to water and food. The mean weight of all animals used was 150 g±3.The animals are randomized into groups according to the treatment schedule as follow:

Group I: Five rats served as control; animals were sacrificed immediately without treatment.

Group II: Five rats received 46.7mg/kg of thiamine as a single oral dose and were sacrificed after one hour.

Group III: Five rats received 70 mg/kg benfotiamine orally as a single dose and were sacrificed after one hour.

GroupIV: Five rats received phenobarbitone intraperitoneally in a single daily dose of 100mg/kg for three days and were sacrificed one hour after treatment with benfotiamine 70 mg/kg as a single oral dose.

Group V: Five rats received cimetidine intraperitoneally in a single daily dose of 30mg/kg for three days and were sacrificed one hour after treatment with benfotiamine 70 mg/kg as a single oral dose.

Blood samples were collected through heart puncture, and serum was prepared for analysis. Kidneys, liver and brain tissues were isolated and tissue homogenates of these organs were prepared according to standard procedures, in order to determine thiamine levels in these organs using HPLC technique (10).After precipitation of protein in the serum and tissue homogenate by using 10% TCA, and centrifugation at 3000 rpm, 20µl aliquots of the supernatant were injected into HPLC at the following condition:

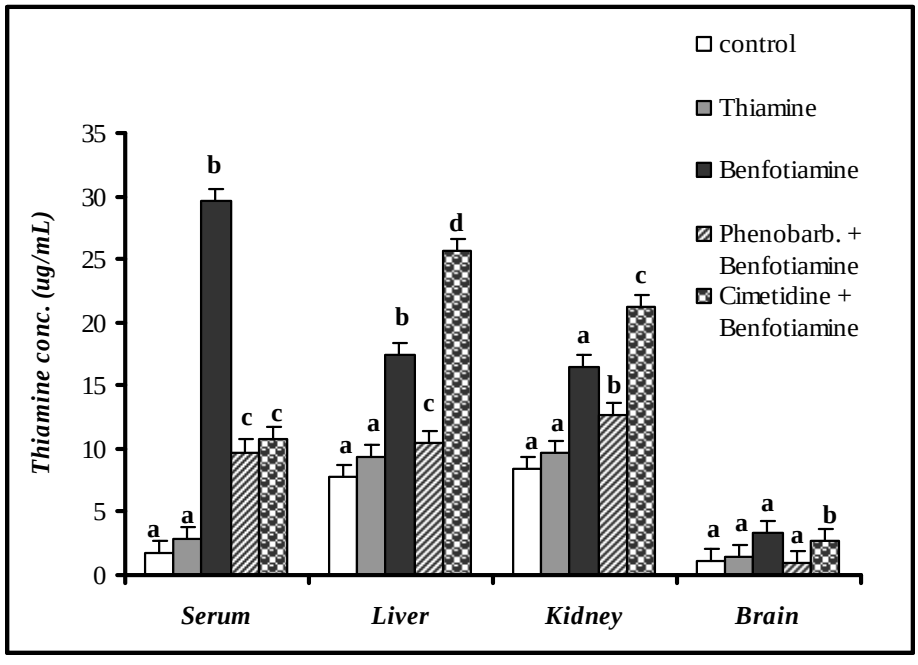
Column: C18, 5µm (4.5x 150mm). Flow rate: 1.0ml/min.Mobile phase: 8:1 0.1N Perchloric acid: Acetonitrile. Isocratic. Detector: UV-detector at λ= 254 nm.

Area under the curve and retention time were compared with those belong to standard authentic sample of thiamine used for this purpose, utilizing ChromGate® Version 3.1 software for personal computer (PC) linked to the HPLC apparatus . The significance of differences between the mean values was calculated using unpaired Student's t-test. P values less than 0.05were considered significant for all data shown in this respect.

Results:

Results presented in table (1) and figure (1) showed that oral administration of thiamine (46.7 mg/kg) did not significantly elevates thiamine levels in serum , liver , kidney and brain ; while orally administered benfotiamine(70 mg/kg) significantly elevates thiamine levels($p < 0.05$) in both serum (17 fold) and liver (2.2 fold) only, while the extent of elevation in other studied compartments (kidney and brain) was non-significant compared to control(fig.1 and table 1)

The results presented in table (1) and figure (1) also indicated that administration of 70 mg/kg benfotiamine orally in phenobarbitone- treated animals (group IV) resulted in significant lower values of thiamine levels in the serum , liver and kidney in comparison to those not- treated with phenobarbitone (group III), while thiamine levels in the brain are not significantly affected . More over, administration of 70mg/kg benfotiamine orally in cimetidine (30mg/kg) treated animals resulted in significantly lower thiamine levels in the serum compared to group III animals ; while thiamine levels in the liver , kidney and brain tissues are significantly ($p<0.05$) elevated compared to this values in the same compartments in group III animals.



(Fig.1) Bar chart comparing the level of thiamine in different compartments in different groups of rats.

Groups	Thiamine level (ug/mL)			
	Serum	***Liver***	***Kidney***	***Brain***
Group I	1.715 ± 0.20 a	7.79 ± 1.86 a	8.4 ± 0.23 a	1.065 ± 0.20 a
Group II	2.83 ± 2.35 a	9.34 ± 3.58 a	9.65 ± 2.79 a	1.432 ± 0.61 a
Group III	29.54 ± 9.61 b	17.42 ± 4.58 b	16.46 ± 9.33 a	3.32 ± 3.46 a
Group IV	9.70 ± 7.28 c	10.42 ± 1.20 c	12.64 ± 1.32 b	0.97 ± 0.27 a
Group V	10.72 ± 5.64 c	25.58 ± 5.24 d	21.22 ± 5.04 c	2.67 ± 1.04 b

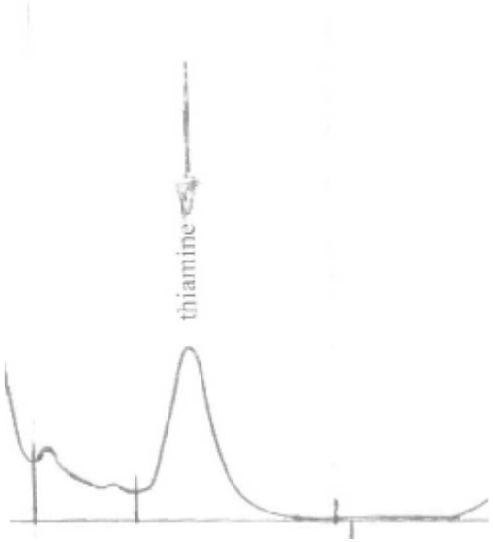
Table (1) shows the level of thiamine in different compartments in different groups of rats.

Values are presented as Mean ± SD.

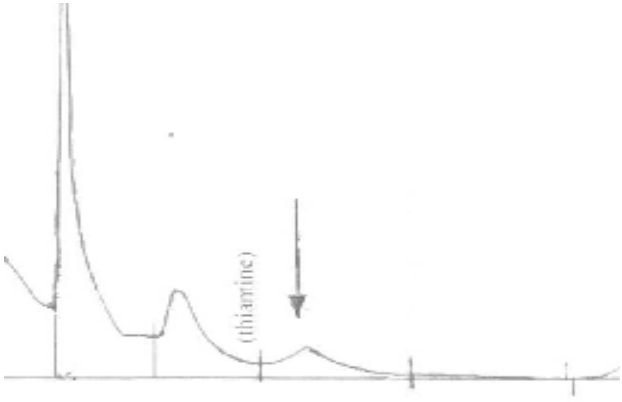
Non-identical superscripts (a, b, c) within the same compartment are considered significantly different, P<0.05.

(Fig. 2) HPLC chromotogram showing serum levels of thiamine in different group of rats. Arrow represent thiamine peak

Thiamine serum level in control group (Group I)



Thiamine serum level in benfotiamine treated group (Group III)



Thiamine serum level in thiamine treated group (Group II)

Discussion

Thiamine is contained in most foods in the biologically active form as thiamine pyrophosphate. For absorption into the body, the phosphate radical must split off on the wall of the intestine by the pyrophosphatases present there [(11)]. For resorption of thiamine, a dose dependent dual transport mechanism is assumed, consisting of active resorption up to administered doses < 2µmol and passive diffusion at higher doses [(12)]. Thiamine is absorbed from the lumen of the small intestine, mainly the jejunum and is transported by the portal circulation to the liver and by the systemic circulation to various tissues in the body. Thiamine is metabolized to thiamine monophosphate (TMP), thiamine pyrophosphate (TPP) and thiamine triphosphate (TTP). Thiamine is phosphorylated directly to thiamine pyrophosphate by thiamine diphosphokinase and thiamine pyrophosphate is dephosphorylated to thiamine monophosphate via thiamine diphosphatase. Approximately 80% in blood is present in erythrocytes as thiamine pyrophosphate (TPP). The transport of thiamine into erythrocytes appears to occur by facilitated diffusion; it enters other cells by an active process. Total thiamine content in the adult body is about 30 mg. Thiamine and its metabolites are mainly excreted by the kidneys [(13)].

The lipid soluble thiamine derivatives known as allithiamines (benfotiamine) in contrast to the thiamine do not appear to be subject to the rate limiting transport mechanism. Benfotiamine is resorbed in proportion to the dose, as owing to its lipid- solubility the substance is not subject to any saturation kinetics, as in the case with thiamine. It is quite clear that benfotiamine is absorbed much well than water –soluble thiamine salts [(14)]. After oral administration of benfotiamine, dephosphorylation by phosphatases to S-benzoylthiamine(SBT) occurs in the intestine. This is better resorbed than water soluble thiamine derivatives, and passes from the circulatory blood into the interior of the cell where the enzymatic debenzoylation to thiamine occurs, which is then converted by thiamine kinase to the active co-enzyme form carboxylase [(15)]. With benfotiamine, higher intracellular concentrations of thiamine and the active co-enzymes are attained than with orally administered water soluble thiamine derivatives [(16)]. The benfotiamine is indeed taken up by cells and tissues more avidly than either thiamine itself or other allithiamines is demonstrated not only by the clinical and experimental studies which early show the superiority of benfotiamine to thiamine in correcting diabetic nerve and kidney dysfunction, but also by direct pharmacokinetic studies documenting it is higher accumulation and retention within cells and tissues after both oral and intravenous administration , and its especially selective incorporation into neural tissue [(17)]. Administration of benfotiamine rather than thiamine gives much higher blood and tissue levels than do equimolar doses of water soluble thiamine derivatives [(18)]. Results in this study show benfotiamine was more bioavilable and bioactive than thiamine supplement , not only in increasing serum thiamine levels , but also raising levels within tissues , these can be agreed with other studies [(19,20)]. Benfotiamine has an excellent tolerability profile and can be taken for long periods without adverse effects [(21)]. The use of benfotiamine in this study cause an increase in thiamine level in all studied compartments , but the level of thiamine in brain seems to be non significantly increase, in spite of lipid solubility of benfotiamine , this can be agreed with other studies [(22,23)], the distribution in brain cells may be required more time than other compartment, and according to my opinion I thought that the levels of thiamine pyrophosphate (monophosphate, diphosphate, triphosphate) will show higher elevation than thiamine itself. It has been observed that the use of phenobarbitone intraperitoneally for three days in a daily dose of 100mg/kg before benfotiamine treatment was associated with significant reduction in thiamin level when compared with benfotiamine alone in case of serum and kidney, while in liver and brain show non significant reduction when compared with benfotiamin alone, may be due to the induction of the enzyme responsible

for metabolism of S-benzoylthiamine (SBT).In addition the use of cimetidine intraperitoneally for five days in a daily dose of 30mg/kg can produce significant elevation in thiamine level in liver and kidney compartment due to the inhibition of the enzymes.

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