ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 3, 2015

ISSN - 0974-2441

Research Article

BIOAVAILABILITY STUDY OF PHYSICAL MIXTURE OF CARBAMAZEPINE AND AMINO ACIDS

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Received: 12 December 2014, Revised and Accepted: 29 December 2014

ABSTRACT

Objective: This study aimed to evaluate the bioavailability of physical mixture (PM) of carbamazepine (CBZ) and amino acids (glycine, alanine, and lysine), includes a parameter t_{max}, C_{max} and AUC_{0-12}.

Methods: PM made by weighing CBZ with amino acids (glycine, alanine, and lysine) equimolar and mix both components with a mortar until a homogeneous mixture. PM obtained was characterized using differential thermal analysis (DTA), Fourier transform infrared (FTIR) and optical microscopy. Bioavailability was conducted after obtaining ethical clearance from Faculty of Veterinary Airlangga University to five New Zealand Rabbits for each treatment. CBZ levels in blood plasma were determined by HPLC analysis method.

Results: The results of the DTA thermogram and infrared spectra showed that CBZ compound mixed with the components of the constituent amino acids. Time achieve maximum levels value (t_{max}) PM of CBZ-GLY, CBZ-ALA, and CBZ-LYS, respectively, for 5.09, 3.85, and 3.93 hours faster than the t_{max} value of CBZ at 6.14 hours. C_{max} value of PM CBZ-LYS at 4.85 mg/mL higher than CBZ at 2.56 mg/mL and AUC_{0-12} value PM CBZ-LYS at 37.31 hrs ug/mL greater than CBZ at 20.59 µg hrs/mL.

Conclusion: From the research can be concluded that PM able to improve the bioavailability of CBZ. The value t_{max} of PM CBZ-amino acids (GLY, ALA, and LYS) faster than CBZ. PM of CBZ-LYS provides C_{max} and AUC_{0-12} value greater than CBZ compounds.

Keywords: Carbamazepine, Glycine, Alanine, Lysine, Physical mixture, Bioavailability.

INTRODUCTION

Carbamazepine (CBZ) is an antiepileptic drug of choice desirable to give effect immediately [1]. CBZ including BCS Class II drug has a low solubility in water and good permeability in the mucous membranes, characterized by slow and erratic bioavailability. Maximum concentration of CBZ in plasma achieved after 4-8 hrs [2]. A drug should be dissolved in a solution aqueous to provide a therapeutic effect. The drugs are low solubility in water, the dissolution is a rate limiting step absorption phase [3]. Increased solubility of poorly soluble drug substance in water can be done through chemical modification and physical modification. Increased solubility can be achieved through modification of its physical properties, among others, can be done with a particle size reduction, crystal habit modification, adding surfactants, complex formation, solid dispersion, and cocrystal [4].

Physical modification of water soluble drug compounds can be done through the establishment of cocrystal by utilizing chemical groups in drug compounds soluble in water with a coformer soluble in water. Interaction of the two components of the compounds can occur through hydrogen bond formation [5] (Fig. 1).

CBZ has the amide group in its molecular structure so that it can interact with the carboxyl group of the amino acid compounds to form hydrogen bonds. By analogy cocrystal formation involving hydrogen bonds in the interaction, in this study made a physical mixture (PM) of CBZ with amino acids. Isadiartuti et al. (2014) study, showed the solubility of CBZ PM with the amino acid glycine, alanine, and lysine did not increase significantly compared to CBZ compounds [6]. Solubility increased of PM associated with changes in the form of polymorph III into dihydrate after CBZ contact with aqueous media to reach equilibrium solid phase and dissolved phase CBZ [7]. Although the solubility of PM CBZ and amino acids did not increase significantly, but dissolution showed a significant increase in the amount of dissolved CBZ for 60 minutes. CBZ polymorphs form change phenomena in aqueous media is reinforced by Kobayashi et al. (2000) study, which showed the intrinsic dissolution form III of CBZ in the water turns into dihydrate form after 2 hrs [8].

One of the factors that affect the bioavailability (*in vivo*) of a drug is the dissolution (*in vitro*). High dissolution will be correlated with higher bioavailability [9]. Therefore, in this study, we want to evaluate the bioavailability of PM of CBZ with amino acids glycine, alanine, and lysine to CBZ, which includes parameter t_{max}, C_{max}, and AUC_{0-12}.

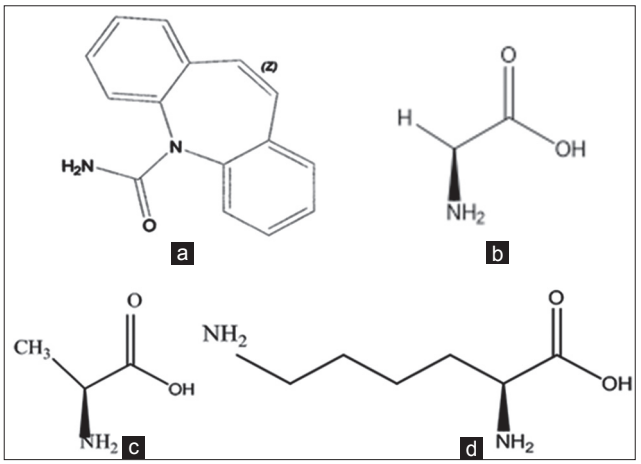

Fig. 1: Molecular structure of carbamazepine (A), glycine (B), alanine (C), lysine (D) [6]

METHODS

Materials

CBZ (*pharmaceutical grade*) was obtained from PT Mersifarma Tirmaku Mercusana Indonesia (Batch No. 09083044). Glysine, alanine, and lysine were obtained from PT Ajinomoto Indonesia. Heparin Sodium (PT Pratapa Nirmala), Na EDTA, methanol p.a (Merck) and distilled water were used.

Method

Preparation of PM CBZ with amino acids

PM of CBZ with amino acids (GLY, ALA, and LYS) were prepared with a 1:1 molar ratio. Both ingredients are mixed until homogeneous. PM obtained is determined by Differential Thermal Analysis (DTA) and Fourier Transform Infra Red Spectroscopy (FTIR) and optical microscope.

DTA

Weighed sample of about 5 mg, inserted into an aluminum sample pan, then placed in a DTA instrument (Mettler Toledo). The heating rate is set at 10°C/minute, with the heating temperature range of 50-300°C. The resulting thermogram profile is then analyzed.

FTIR

Each sample was weighed as much as 2 mg, then added with KBr powder pro spectrophotometry that has been dried as much as 300 mg. Then crushed in a mortar mix until homogeneous, put in a KBr disc creation tools, compressed by hydraulic pressure to obtain a transparent disc. Then the disc is placed in the sample holder and recorded instrument (Jasco FTIR 5300). Samples were observed in the absorption band or wave number 4000-450 cm^{-1}.

Optical microscope

PM of CBZ with amino acids (glycine, alanine, and lysine) in dried crystals upon exposure to water obtained were observed in an optical microscope with a magnification of 40 and compared with the initial compound CBZ.

Bioavailability study

Bioavailability study conducted on the five male New Zealand Rabbit, weight 2.0 ± 0.5 kg, aged 1-1.5 years for each treatment. Bioavailability is done after permission has been obtained from Etics Committee of the Faculty of Veterinary Medicine, University of Airlangga (No. 245 KE). Twelve hours prior to the study, the rabbits were put in fast but given the excessive water. All rabbits get the dosage orally in a single dose equivalent of 120 mg CBZ and suspended in 10 mL of distilled water. Blood samples were collected from the rabbit marginal vein taken by individual venous puncture before dosing (0 hrs) and on the 0.5, 1, 2, 3, 4, 5, 7, 9, and 12 hrs. Blood samples were centrifuged for 30 minutes with the speed of 3500 rpm to separated plasma and stored at −20°C prior to analysis by HPLC method (UFLC Shimadzu LC-20AD).

RESULTS AND DISCUSSION

Characterization of the PM CBZ with the amino acid glycine, alanine, and lysine was analyzed by DTA and FTIR. Besides in PM have been contacting with aqueous media, dried solid form observed with an optical microscope magnification ×40.

DTA thermogram of CBZ and PM of CBZ with glycine, alanine, and lysine amino acids with equimolar ratio can be seen in Fig. 2. The sharp endothermic peak of CBZ at a temperature of 192.6°C looks to shift toward lower temperatures in the thermogram of PM. DTA thermogram shows a shift other than the PM endothermic peak of CBZ are also visible above the endothermic peak temperature of 200°C from its constituent amino acids. PM melting point shift lower due the interaction of two components [10].

Fig. 3 shown, FTIR of CBZ spectra give characteristic absorption bands form III of CBZ at wave number 3465 cm^{-1} (-NH stretching), 1677 cm^{-1} (-C = O stretching), 1605 and 1594 cm^{-1} (range of -C = C - and -C = O vibration and -NH deformation), and 1384 cm^{-1} (-C = N bond) [11-13]. The FTIR spectra of the PM show peaks that indicate the presence of functional groups CBZ and amino acids [6].

Micrograph of a PM of CBZ-GLY, CBZ-ALA, and CBZ-LYS which have contacts water compared with CBZ in the same condition can be seen in Fig. 4. Changes in the crystal habit of lamelar into needle shape

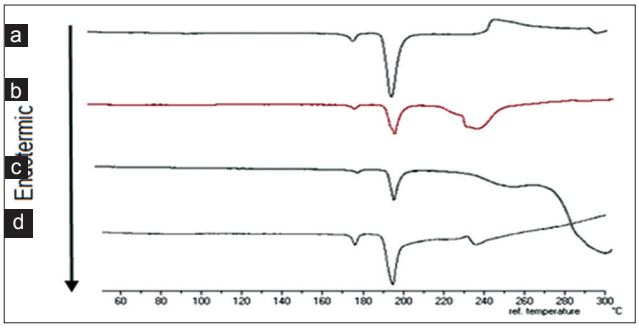

Fig. 2: DTA Thermogram of CBZ (a), PM CBZ-GLY (b), PM CBZ-ALA (c), and PM CBZ-LYS (d) [6]

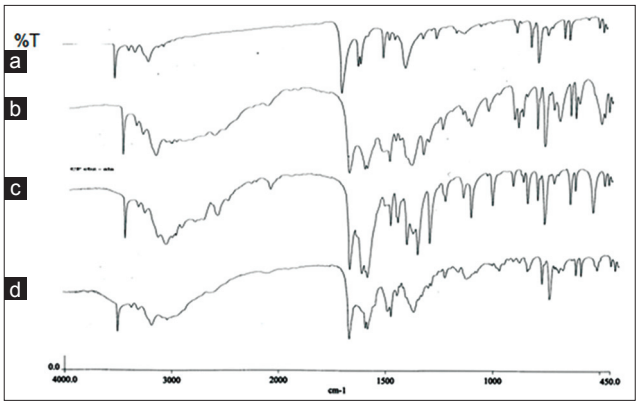

Fig. 3: Infrared spectra of CBZ (a) PM CBZ-GLY (b), PM CBZ-ALA (c), and PM CBZ-LYS (d) [6]

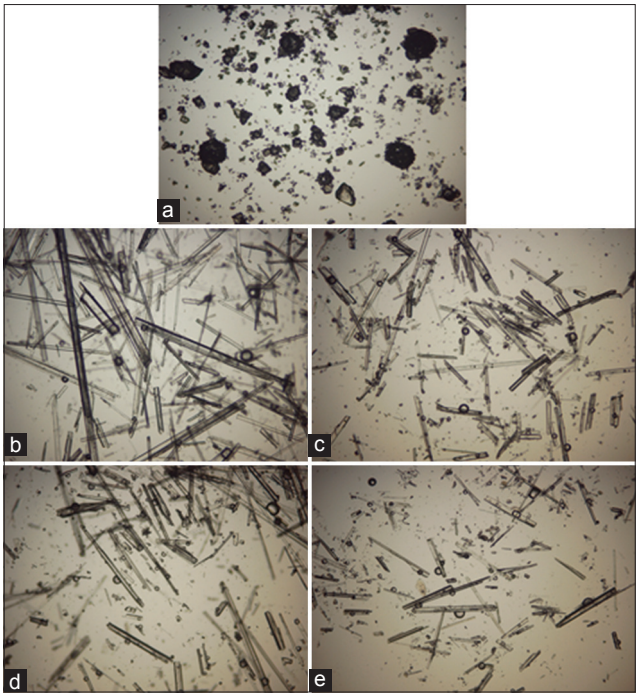

Fig. 4: Micrograph of CBZ initially compound (a), dried crystals upon exposure to water of CBZ (b), PM CBZ-GLY (c), PM CBZ-ALA (d), and PM CBZ-LYS (e) with optical microscope magnification × 40

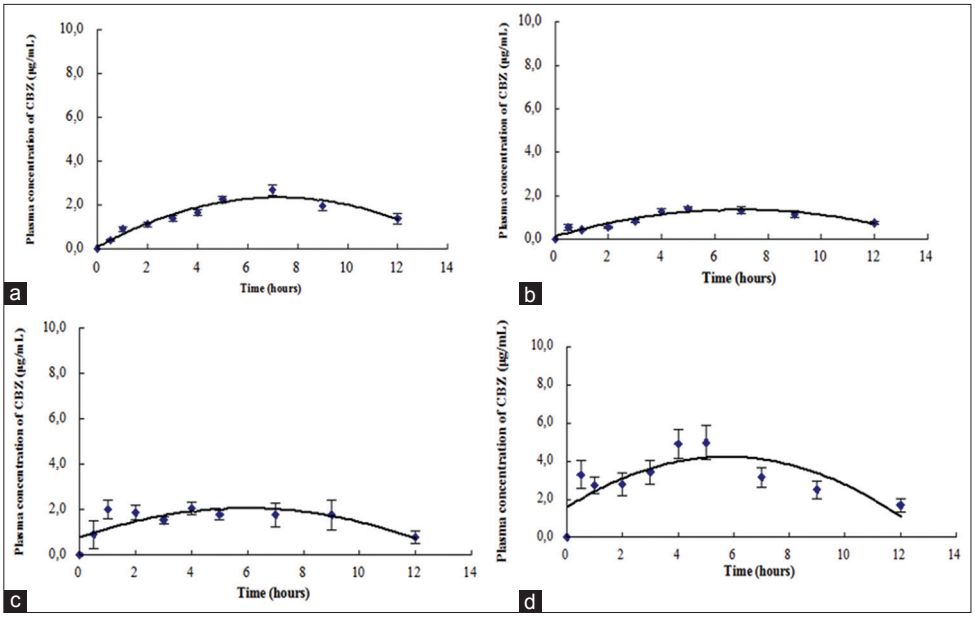

Fig. 5. Bioavailability profile of CBZ (a), PM CBZ-GLY (b), PM CBZ-ALA (c), PM CBZ-LYS (d) (Mean ± standard error (n=5))

Table 1: Dissolution rate constant (hrs^{-1}) CBZ and PM of carbamazepine and amino acids in the distilled water medium pH 6.8±0.5 at a temperature of 37±0.5°C (n=3)

Substance	k_{dis} (hrs^{-1})
CBZ	1.13
PM CBZ-GLY	1.96
PM CBZ-ALA	1.86
PM CBZ-LYS	2.73

PM: Physical mixture, CBZ: Carbamazepine

that happens to CBZ and PM are exposed to water which indicates CBZ dihydrate was form [14]. The third PM has a smaller crystal size than CBZ. PM of CBZ-LYS has the smallest size among the four forms dihydrate. This is because the amino acids of lysine have the greatest water solubility compared to glycine and alanine. In the process of the formation of the dihydrate crystals in water, lysine molecules urged the formation of CBZ dihydrate crystals to produce crystals smallest of the four forms of the dihydrate were observed. PM of dried crystal CBZ-LYS size smaller than CBZ and PM of CBZ-GLY or CBZ-ALA cause the surface area in contact with the solvent water is greater (Sinko, 2011) so as to explain the solubility of PM CBZ-LYS greater than CBZ and PM of CBZ-GLY or CBZ-ALA [6].

From Isadiartuti *et al*. (2014) study, although the solubility of CBZ PM of amino acids did not increase significantly compared to the initial compound CBZ, the dissolution of the PM of CBZ with amino acids showed a significant increase. Table 1 shows the value of rate constants dissolution (k_{dis}) PM CBZ-LYS > PM CBZ-GLY = PM CBZ-ALA > CBZ. respectively for 2.73, 1.96, 1.86, and 1.13 (hrs^{-1}).

In the early dissolution of CBZ interaction with amino acids to form hydrogen bonds. Hydrogen bonding interactions that occur at the beginning of the dissolution causes increased dissolution of PM significantly different from CBZ compound. After 60 minutes, hydrogen bonds formed between CBZ and amino acids components separated, so the dissolution decreased. The phenomenon that occurs according to research conducted Kobayashi *et al*. (2000) [8].

Dissolution is a kinetic process that depends on the time and describes the final stage before a drug is absorbed and delivery pharmacological effect [9]. Compounds that have better dissolution will be removed completely so as to produce plasma drug levels are

Table 2: Pharmacokinetic parameters of CBZ after orally administration of CBZ, PM CBZ-GLY, PM CBZ-ALA, and PM CBZ-LYS to rabbit (n=5)

	CBZ	PM CBZ-GLY	PM CBZ-ALA	PM CBZ-LYS
k_a (hrs^{-1})	0.20	0.30	0.48	0.44
k_{el} (hrs^{-1})	0.13	0.12	0.12	0.13
$t_{1/2}$ (hrs^{-1})	5.33	5.78	5.78	5.33
T_{maks} (hrs)	6.14	5.09	3.85	3.93
C_{maks} (µg/mL)	2.56	1.38	2.04	4.85
AUC_{0-12} (µghrs/mL)	20.59	11.88	18.08	37.31

PM: Physical mixture, CBZ: Carbamazepine

higher. Therefore, dissolution may affect onset, intensity, and duration of therapeutic response and control all aspects of bioavailability [3].

The mean concentration plasma-time curve states bioavailability profile CBZ and PM of CBZ with the amino acid after oral administration can be seen in Fig. 5. The results show inter-subject variability was great although the sample population of experimental animals is limited to the requirements of the type, age, weight bodies and feed. These conditions in accordance to the literature which states that CBZ has a slow characteristic and erratic bioavailability [2]. Based on the bioavailability profile of each treatment was calculated pharmacokinetic parameters such as can be seen in Table 2.

From the table can be seen that the PM of CBZ -amino acids have absorption rate constant (k_a) greater than the value k_a of CBZ (PM CBZ-ALA > PM CBZ-LYS > PM CBZ-GLY > CBZ). The greater value of k_{dis} PM causes the availability of CBZ to be absorbed in the gastrointestinal tract is greater so that it can act as a driving force gradient for the absorption process, especially when it involves the absorption of passive diffusion mechanism.

Elimination rate constant (k_{el}) of PM did not differ significantly compared to the value of k CBZ. This is due to CBZ compounds in the body that will separate from the PM, so that the process of elimination of PM as compound CBZ. Based on the value of the half-life ($t_{1/2}$) of each treatment can be predicted period of drug substance in the body. The half-life of a compound is determined by its k value. Compounds which

have large value of k will have a short half-life [9]. From the calculations, the half-life of PM does not differ from the half- life CBZ.

Time to achieve maximum levels (t_{max}) PM faster than t_{max} CBZ (PM CBZ-ALA > PM CBZ-LYS > PM CBZ-GLY > CBZ). Total CBZ is dissolved in the gastrointestinal tract to cause faster absorption of CBZ, therefore, shorten the time of CBZ to reach maximum levels in the blood. The value of k_a and t_{max} was not correlated with the value of the maximum levels of CBZ (C_{max}) in the blood at PM CBZ-GLY and PM CBZ-ALA. This condition, that accur due to the absorbtion process is not only influence by how the physicochemical properties compound are absorbed, but is also influenced by the anatomy structure and physiology of the gastrointestinal tract conditions. These conditions have an impact on the AUC_{0-12} value of PM CBZ-GLY, which is lower than CBZ and, therefore, requires further study.

CONCLUSION

From the research, it can be concluded that PM able to improve the bioavailability of CBZ. The value t_{max} of physical mixture CBZ-amino acids (GLY, ALA, and LYS) is faster than CBZ. Physical mixture of CBZ-LYS provides C_{max} and AUC_{0-12} value are greater than CBZ compounds.

ACKNOWLEDGMENT

The author would like to thank the Directorate General of Higher Education, Ministry of Education and Culture Republic Indonesia through BOPTN of Airlangga University 2014 for the financial support this research.

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