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Potential Drug Interactions of a Polyherbal Antihypertensive Mixture in Ghana

George A. Koffuor*; EricWoode and Cynthia AmaningDanquah

Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST

ABSTRACT

Although most herbal medical practitioners claim safety with the concomitant use of both herbal and orthodox medicines, the possibility of drug interaction cannot be ruled out. This study therefore aims at finding the effect of a commonly usedpolyherbalantihypertensive in Ghana onpentobarbitone-induced sleeping time and cytochrome P450, liver microsomal enzyme, in male Imprint Control Region mice for possible drug interactions. Data obtained was analyzed using Graph Pad Prism (version 5) for windows. Results obtained indicated a dose dependent significant reduction ($P \le 0.05$ -0.001) in the onset and duration of pentobarbitone-induced sleep in mice treated with the polyherbalantihypertensive which was similar to those treated with diazepam. The CNS depressant activity of PHA cannot be ruled out due to the fact that it decreases significantly the onset of pentobarbitone-induced sleep and prolongs the duration of sleep. This activity could be due to interference with barbiturate enzymatic metabolism. Compared to the control, there was also a very significant decrease ($P \leq 0.001$) in the cytochrome P450 level in mice treated with ketoconazoleand the antihypertensive product. This will affect the liver's ability to effectively metabolize pentobarbitone. Since this study has shown that PHA inhibits CYP450 enzymes, it is advised that caution should be taken when the polyherbal antihypertensive product is used concomitantly with drugs sedative-hypnotics and other drugs that are metabolized by cytochrome P450 since it could potentiate their activity.

Key words: Pentobarbitone-induced sleep, cytochrome P450, ketoconazole, CNS depressant, righting reflex.

INTRODUCTION

In Ghana, the majority of patients taking herbal medicines for the management of hypertension are also on some allopathic medicines. Although most herbal medical practitioners claim safety

with the concomitant use of both herbal and orthodox medicines, the possibility of drug interaction cannot be ruled out. The larger proportion of these patients do not inform their health care givers as to the use of herbs with allopathic medicines, and most physicians and pharmacists do not enquire about herb use of their patients, probably believing that there is no such need Studies have shown that consulting with physicians does not prevent patients from co-administering prescription medicines and herbal medicines [1-3]. More than one-third of the ambulatory hypertensive patients interviewed in a Nigerian hospital were found to be using herbal medicine [4]. Although almost all the plants used by the respondents have proven ethnopharmacological and folkloric uses, this practice could be potentially harmful as far as the health of the individual is concerned.

Although some of the biological activities, such as reduction of blood cholesterol, antioxidant and blood-pressure-lowering properties, possessed by some of the herbs may be beneficial, its concomitant administration with allopathic medicines needs to be monitored with adjustment of doses, if necessary, and decisions on suitability of use of herbs can then be made with the patient, if need be. The latter may be necessary especially if an antihypertensive agent is being concomitantly administered with a herb that possesses blood pressure-lowering effect.

Quite a number of physicians and pharmacists believe that there may be drug-herb interactions, [5, 6] but not much effort is made to investigate patients' herb use. This oversight might have contributed to some of the incidences of side effects experienced as a result of herb use by some patients, which could have been prevented if they had been advised appropriately e.g. gingko interacts with aspirin with the potential of increased risk of bleeding, ginseng may interact (unpredictable) with warfarin hence the concomitant use may lead to a risk of prolonged bleeding, and St. John's wort may decrease theophylline's plasma concentration, thereby reducing its therapeutic effect. It is in this light that the effect of PHA on; pentobarbitone-induced and cytochrome P450 (liver microsomal enzyme) sleeping time is being studied for possible drug interactions.

MATERIALS AND METHODS

Animals and Husbandry

Male Imprint Control Region (ICR) mice at 3-4 weeks of age (weighing 30-35g) were obtained from the Department of Pharmacology, KNUST, and animal house and acclimated for 2 weeks prior to initiation of dosing. During this period, mice were observed (physical; in-life) daily and weighed. At initiation of treatment, animals were approximately 5 weeks old. Individual weights of mice placed on test were within \pm 30% of the mean weight for each sex. All mice were examined during the acclimation period to confirm suitability for study and those considered unsuitable were eliminated.

Animals were housed in stainless steel, wire mesh cages during the acclimation and the experimental periods. The mice were kept under ambient light/dark cycle, room temperature and relative humidity. The animal had free access to pelleted mice chow (GAFCO, Tema, Ghana) and water daily.

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This study was conducted in 2010 at the Department of Pharmacology, KNUST in compliance with all appropriate parts of the Animal Welfare Act Regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112–36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426–6505 effective March 18, 1991.

Chemicals Used

Diazepamand caffeine (Sigma and Aldrich, St. Louis MO, USA)was used in this study as a reference CNS depressant and stimulantrespectively. Sodium pentobarbital (BDH chemicals Ltd, Poole, England)was used to induce sleep in experimental animals. Ketoconazole (Janssen Pharmaceutical Products, Titusville, NJ) was employed as a liver microsomal enzyme inhibitor and phenobarbitone(BDH chemicals Ltd, Poole, England) anenzyme inducer.Sucrose, calcium chloride, sodium dithionite, sulfuric acid obtained from BDH chemicals Ltd, Poole, England, and formic acid from Fisons Laboratory Reagent, Longborough, England were reagents that were employed in the cytochrome P450 assay.

The Polyherbal Product (PHA) and Dosing

A prepackage preparation made from leaves and bark of *Perseaamericana*, and *Vernoniaamygdalina* referred to in this study as PHA is the product under study. Dosing of PHA was based on the manufacturer recommendation which was calculated to be 55 mg/kg/day. ICR mice were randomly distributed into a control group and treatment groups and received either vehicle or PHA by gavage. Dosing was a single event at a volume of 10 ml/kg body weight. Individual dose volumes were calculated based on the animal's most recent recorded body weight. The oral route of administration was used because it is the intended human exposure route.

Pentobarbitone-Induced Sleeping Time

ICR mice were grouped into six with 5 animals per group and received vehicle, diazepam (0.08 mg/kg, i.p), caffeine (100 mg/kg, i.p) or PHA (36, 72 and 180 mg/kg, p.o). Thirty minutes afterwards each animal was injected with sodium pentobarbital (40 mg/kg, i.p.). The time which elapsed from the injection to the loss of the righting reflex (onset of sleep) and the times from the loss of rightness reflex to awakening (duration of sleeping) were registered for each animal.

Cytochrome P450 Assay

ICR mice were groups into four with 10 animals per group and received vehicle or ketoconazole (80 mg/kg, p.o), phenobarbitone (100 mg/kg, p.o), or PHA (36, 72 and 180 mg/kg. p.o) by gavage. Dosing was once daily for 14 days at a volume of 10 ml/kg body weight.

Preparation of Tissue Homogenates

After the two week treatment period, the mice were euthanized and their livers were rapidly excised and immediately placed in ice-cold 0.25 M sucrose to wash off excess blood and to cool the liver. The liver was then blotted dry, weighed and added to four times its weight of 0.25 M sucrose, to obtain a 20 % (w/v) homogenate. The liver was finely chopped with scissors and homogenized with Potter-Elvehjem homogenizer (Omni international, USA) with a Teflon pestle. The homogenate was centrifuged in a Mikro 220Rrefrigerated centrifuge (Hettich, Zentrifugen, Hattingin, Germany) to isolate subcellular fractions.

Preparation of Post-Mitochrondrial Supernatant

Post-mitochondrial supernatant was prepared by centrifuging the homogenate in a refrigerated centrifuge at 3000 g for 15 minutes to pellet intact cells, cell debris, nuclei and mitochondria. The resultant supernatant (the post-mitochondrial supernatant) was carefully decanted. This contains the microsomal plus soluble (cell sap) fractions of the cell.

Preparation of Subcellular Tissue Fractions

Aliquot (10 ml) of post-mitochondrial supernatant were mixed with 88 mM CaCl₂, such that 0.1 ml of 88 mM CaCl₂ was added per ml of supernatant (final CaCl₂ concentration is 8 mM) and left to stand on ice for 5 minutes, with occasional gentle swirling. The mixture was then centrifuged at 6000 g for 15 minutes, the supernatant discarded and the pellet resuspended by homogenization in 5 ml of 0.1 M Tris buffer, pH 7.4, yielding the microsomal suspension.

Determination of CYP450

A 0.1 ml of the tissue samples were diluted in 10 ml phosphate buffer, pH 7.4 containing 20 %(v/v) glycerol to approximately 2 mg/ml. A 2 ml of the diluted samples were then added to sample cuvette and a baseline absorbance recorded between 400-500 nm by a WPA S105 spectrophotometer (Saffron Walden, England). A few grains of solid sodium dithionite were added to the sample cuvette with gentle stirring and the cuvette is bubbled with carbon monoxide (produced by mixing 12.5 ml concentration sulfuric acid and 25 ml formic acid in an infusion bottle which was plugged with a perforated rubber stopper attached to a giving set) for approximately 30 s. The absorbance was again recorded and cytochrome P450 content was calculated using Beer's law and assuming a cuvette path length of 1 cm:

Cytochrome P450 (nmol/ml diluted)= <u>Absorbance difference (nm) x 1000</u> Extinction coefficient mM-¹cm-¹

The reduced, carbon monoxide difference spectrum of cytochrome P450 absorbs maximally at around 450 nm and the extinction coefficient for the wavelength couple 450-490 nm has been accurately determined to be 91 mM-¹cm-¹. The procedure was repeated three times.

Statistical Analysis

The observations are presented as mean \pm SEM. Significant differences among means of the group were determined by one-way ANOVA using Graph Pad Prism for windows version 5.00 (Graph Pad Software, San Diego, CA, USA). Significant differences between pairs of groups were calculated using the Turkey's multiple comparisons test with level of significance set at P \leq 0.05.

RESULTS

Pentobarbitone-induced Sleeping Time

There was a dose dependent significant reduction ($P \le 0.05$ -0.001) in the onset and duration of sleep in mice treated with PHA. This effect was similar ($P \le 0.001$) to animals treated with diazepam. The onset and duration of sleep for the caffeine treated group was not significantly different from that of the vehicle treated group (control).



Treatment groups





Treatment groups

Figure 2: The effect of diazepam, caffeine and PHA on the duration of sleep induced by pentobarbitone (40 mg/kg) in ICR mice. For significant differences compared to the control: *** implies $P \le 0.001$, * implies $P \le 0.05$, ns implies P > 0.05. N=5



Figure 3: The effect of phenobarbitone, ketoconazole, and PHA on the total CYP450 of ICR mice after a twoweek treatment period. For significant increments compared to the control: ** implies $P \le 0.01$. For significant decrements compared to the control: $\dagger \dagger \dagger \dagger$ implies $P \le 0.001$; ns implies P > 0.05. N=3

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Cytochrome P450 Assay

Compared to the control, there was a very significant decrease ($P \le 0.001$) in the CYP450 level in ICR mice treated with ketoconazole and the antihypertensive product. In the phenobarbitone treated group however, there was a significant increase ($P \le 0.01$) in cytochrome P450 levels.

DISCUSSION

The CNS depressant activity of PHAcannot be ruled out due to the fact that it decreases significantly the onset of pentobarbitone-induced sleep and prolongs the duration of sleep. This activity could be due to interference with barbiturate enzymatic metabolism. Through secondary hepatic metabolic inactivation barbiturates lose their affinity for the GABA receptor complex and thus exhibit CNS depressant activity [7]. Therefore for the CNS effect to persists on concomitant administration means that there is a possible drug interaction, hence the determination of its effect on CYP450 enzyme in the liver.

The very significant reduction in CYP450 levels caused by ketoconazole shows that there is a significant inhibition of the enzyme in the mice. Ketoconazole is known to be a cytochrome P450 enzyme inhibitor [8]. Specifically, it inhibits the CYP3A subfamily; the most abundant cytochrome enzymes in humans which account for 30 % of the cytochrome P450 enzymes in the liver [9]and are also substantially expressed (about 70 %) in the intestines. Members of this subfamily are involved in many clinically important drug interactions [10] hence an inhibition of the enzyme has a lot of clinical implications. A similar reduction in CYP450 concentration was seen with the polyherbalantihypertensive product. The product therefore could possibly be inhibiting CPY450 activity.

The enzyme inhibition activity of the polyherbal antihypertensive, which is comparable to ketoconazole, will affect the liver's ability to effectively metabolize most compounds in the human body. The inhibitory property could possibly be achieved by binding tightly to CYP450 heme iron [11] hence reducing the levels of active enzymes to metabolize xenobiotics. There is therefore the possibility of drug interaction when taken with anti-arhythmics such as quinidine and anti-diabetics such as sulphonylureas by causing high plasma concentration leading to toxicity. Co-administration of the product with anti-HIV protease inhibitors could reduce the clearance of the protease inhibitor and resultant toxicity [12]. This herbal antihypertensive is most often taken concurrently with orthodox antihypertensives by patients. The metabolism and clearance of the orthodox antihypertensives are reduced by the extract resulting in high plasma concentrations with enhanced hypotensive effect.

CONCLUSION

This study has shown that PHA shortens the onset of sleep and prolongs the duration of sleep possible by the fact that it inhibits CYP450 enzymes. It is advised that caution should be taken when the antihypertensive product is used concomitantly with orthodox antihypertensive preparations and sedative-hypnotics since it could potentiate their activity.

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