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RESEARCH ARTICLE

EVALUATION OF CYTOCHROME P450 MICROSOMAL ENZYME INDUCING ABILITY OF BARBITURATES IN MALE ALBINO RATS

***,[1]Onuigbo, H. N., [2]Nwobodo, N. N. and [3]Onuigbo, I. B.**

[1]Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu, Nigeria
[2]Department of Pharmacology and Therapeutics, Faculty of Medicine,
Ebonyi State University, Abakaliki, Nigeria
[3]Department of Surgery, Enugu State University Teaching Hospital, Park lane, Enugu, Nigeria

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ABSTRACT

Barbiturates are hypnotic (sleep-inducing) drugs and notable drugs of abuse. They are extremely useful in clinical practice and are commonly prescribed as treatment for a variety of conditions. Barbiturates are known hepatic enzyme inducers and have significant stimulatory effects on the cytochrome P450 enzyme family. This study was aimed at evaluating the degree of cytochrome P450 enzyme family stimulation in a standard prescribed treatment regimen with a view to ascertaining changes in hepatic mitochondrial protein as an indicator of the drug adverse effect. Parenteral formulation of the drug was administered to the experimental rats in a simple randomized study after 7days of adequate acclamatization. The control animals received placebo treatment. All the animals were sacrificed after completing treatment. Protein status of each animal was assayed in the mitochondrial fraction of the liver while the stimulatory capacity of the drug was assayed in the microsomal fraction. Results revealed that mean protein status of the experimental animals did not differ significantly compared with the control animals ($p>0.05$). However, there was a statistically significant difference ($p<0.05$) on the activity of the cytochrome P450 enzyme family between the test and control animals. Hence, the need for a large scale study involving human subjects considering the widespread use of the drug in clinical medicine.

INTRODUCTION

Disease complexity largely influences the number of drugs administered concurrently or sequentially to patients in the hospitals and clinics. Two or more drugs may be prescribed to patients to be taken once leading to potential drug interaction. Drug interaction may result in decreased potency, increased adverse effects or enhanced toxicity of a drug. One drug may modify the metabolism of another by inducing or inhibiting the metabolic enzymes responsible for its metabolism. Metabolic enzyme induction or inhibition leads respectively to reduced or increased half–life of the second drug with the attendant consequences. Drug interaction may make chronic diseases like high blood pressure, diabetes or epilepsy more difficult to control. The effect of interaction between grapefruit juice and calcium channel blockers such as felodipine and nifedipine has been reported (Bailey *et al.,* 1994). Phenobarbital belongs to a group of drugs known as barbiturates, which are derivatives of 2,4,6-trioxohexahydropyrimidine which is the strongest inducer of metabolic enzymes among the barbiturates. It has very good ultraviolet absorption properties which makes its measurement in biological materials very easy with ultraviolet spectrophotometer. Barbituric acid (barbiturate) is a malonylurea obtained by combining malonic acid with urea.There are many individual drugs in this group which are chemically similar (Fiereck and Tietz, 1971). They are extremely useful in clinical medicine where they are commonly prescribed as treatment for a variety of conditions including insomnia, seizure disorders, sedation, induction and maintenance of anesthesia. They stimulate metabolism of other drugs used concomitantly and thus reduce their half-life. Barbiturates accelerate metabolism of coumarins, griseofulvin, chloropromazine, chloramphenicol, nitrofurantoin, sulfonamide, salicylate, vitamin D, sex hormones and oral contraceptive agents; reducing their blood levels sometimes below therapeutic level. Conversely, microsomal enzyme activities decrease rapidly after discontinuation of barbiturates and results in increased pharmacologic response and toxic effects of drugs involved. The objective of this study, therefore, was to evaluate the ability of barbiturates particularly phenobarbital in inducing cytochrome P450 microsomal enzyme system in drug metabolism, as a pre-requisite for better understanding of underlying mechanisms of drug interactions and toxicity.

Corresponding author: Onuigbo, H. N.
Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu, Nigeria.

MATERIALS AND METHODS

Twenty healthy male albino rats were used for the study and categorized into 10 (test) and 10 (control) respectively, in accordance with ethical standards for biomedical research on laboratory animals. The animals were pooled from the University's animal house. They were well maintained in the laboratory for 7 days before the experiment. The weights of all the animals were measured before and after treatment. The treatment was given once daily for three consecutive days. On the fourth day, all the animals were sacrificed and carefully dissected. The liver mass of each animal was measured. Thereafter, each liver was homogenized separately with an electric blender. Each homogenate was divided into two equal parts. The first part was ultra-centrifuged at 20,000 revolutions per minute (rpm) in equal volume of normal saline (9mg sodium chloride) for five minutes. The supernatant was carefully separated out and the total protein content assayed using Biuret method. The microsomal fraction was isolated from the second part of the homogenate by centrifuging the tissue in 0.25M sucrose at 600 rpm for 10 minutes, first to remove nuclei and cell debris. The supernatant was then ultra-centrifuged at 100,000rpm for 60 minutes. The deposit obtained was the microsomal fraction. It was re-suspended in equal volume of normal saline. 1ml of each suspension was incubated in paranitrophenol phosphate at room temperature for 30 minutes for colour development. The intensity of the colour developed in each tube was measured spectrophotometrically at 420nm. Data analysis was carried out using Graphpad version 4.0 statistical software and results displayed in tabular format. Statistical test of significance determined using two paired Student t-test at 5% significance level, $p<0.05$ considered significant. Data were tested for normality prior to analysis for baseline comparison. Data of the two groups compared showed normal distribution.

RESULTS AND DISCUSSION

The anthropometric and biochemical characteristics of the study animals were as displayed in Table 1. There was no reported adverse reaction following barbiturate administration on the test animals. The Phenobarbital dosing of 0.201mg/g was safe and well tolerated by the test animals. Comparison of the enzyme activities of the test animals (n = 10) with the control animals (n = 10) by Student t–test showed a significant difference ($p<0.05$). Post–treatment baseline liver weight and total protein values of the test animals did not differ significantly from that of the control animals ($p>0.05$).

Table 1. Comparison of Anthropometric and Biochemical Parameters in Test and Control Animals

Parameters	Test Mean(SD)	Control Mean(SD)	P-Value
Number of Animals	10	10	-
Body Weight (g)	197(29.08)	196(29.14)	p>0.05
Dose of Phenobarbitone (mg/g)	0.201(0.03)	0(0)	p>0.05
Normal Saline (ml)	0(0)	0.2(0.03)	p>0.05
Weight of the Liver (g)	36(8.76)	38.7(13.1)	p>0.05
Protein (g)	5.55(1.49)	5.47(1.29)	p>0.05
Enzyme Activity (iu/L)	15(6.47)	4.77(0.9)	P<0.05

The therapeutic effects of barbiturates are mediated by major inhibitory neurotransmitter, Gama-Aminobutyric Acid (GABA) and the liver metabolism largely by oxidation. This study demonstrated a statistically significant ($p<0.05$) stimulation of cytochrome P450 microsomal monoxygenase system by daily administration of phenobarbital for three consecutive days. Previous predictive simulation of *in-vivo* stimulation profiles from i*n-vitro* data suggests that cytochrome P450 stimulation is enhanced by gene transcription following prolonged exposure to stimulating agents. This however, neither reflected in the liver mass measured after treatment nor in the protein values. At present the consequences of the stimulations are as postulated by the following hypotheses: increased rate of metabolism, enhanced oral first-pass metabolism with reduced bioavailability or corresponding decrease in the drug plasma concentration; all of which are known to reduce drug exposure to the target site (Wilkinson and Shard, 1975). By contrast, for drugs that are metabolized to an active or reactive metabolite, stimulation may be associated with increased drug response or toxicity respectively. The significant difference ($p<0.05$) shown by the activities of the enzymes corroborated previous reports.

The microsomal monoxygenase enzyme system has been known to be significantly induced or inhibited by xenobiotics (Czekaj and Nowaczyk-Dura, 1996, 1999; Czekaj *et al.,* 2010). A number of genes are activated in the liver by phenobarbitone, culminating in pleiotropic effects in the liver, involving increase in hepatic mass, proliferation of smooth endoplasmic reticulum, increased activity of phase II enzymes (aldehyde dehydrogenase, *S*-glutathione transferase, uridine diphosphoglucoronyl transferase) and enzymes involved in heme synthesis and lipid metabolism (Waxman and Azaroff, 1992; Frueh *et al*., 1997). A large group of xenobiotic inducers, known as PB (phenobarbital)-like inducers of which phenobarbital is the prototype, regulate gene transcription. PB-like inducers other than barbiturates include carbamazepine, phenytoin, cotrimoxazole and cyclophosphamide (Smith *et al*., 1993; Honkakoski *et al*., 1998; Chang *et al*., 1997). The precise mechanism of cytochrome P450 induction by phenobarbital remains unclear, moreso, considering the influence of phenobarbital on several cytochrome P450 families, particularly CYP 2A, 2B, 2C and CYP 3A (Denison and Whitlock, 1995; Dogra *et al*., 1997; Whitlock and Denison, 1995). Indeed, the multiplicity of interactions between PB-like inducers and their target genes further complicate determination of the underlying mechanism of PB-dependent induction, that is whether receptor mediated or non- specific.

Several cases involving stimulation resulting to increased metabolic rate, may require increased dosage adjustment of the affected drug to achieve the desired therapeutic effect. The therapeutic risk associated with metabolic stimulation is most critical when administration of the stimulating agent is stopped while still maintaining same dosage of the second drug. In this case, as the stimulating effect wears off, plasma concentration of the second drug will rise, unless the dosage is reduced, with an increase in the potentials for toxic effects. Moreover, self-induction for a drug taken for a long time is one of the major causes of drug tolerance. Hence, co-application of drugs which have stimulating (inducing) ability on monooxygenase enzyme

system should be done with balanced clinical judgment to avert adverse effects. In conclusion, the results from this small population of animals is by no means conclusive and this underscores the need for more extensive studies involving human subjects.

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