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Spectrofluorimetric measurement of the induction level of rat hepatic glutathione transferase activity toward mono-bromo-bimane with drug metabolizing inducers.

Yakubu SI[1*], Yakasai I A [2] and Musa A [2]

[1]Department of Clinical Pharmacy and Pharmacy Administration, University of Maiduguri, Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria.

ABSTRACT

Studies have demonstrated that monobromobimane, a non-fluorescent compound reacts chemically with glutathione (GSH) to produce a fluorescent glutathione conjugate. This conjugation of glutathione with monobromobimane can be catalyzed by glutathione transferase. Titrimetric, colorimetric, spectrophotometric, high performance liquid chromatography or radioisotopic techniques of analysis have been used in the study of glutathione and glutathione transferase activity. We have in this study used spectroflourimetric assay method, an alternative technique of analysis, to examine the inducibility of rat hepatic glutathione transferase activity toward monobromobimane with microsomal drug metabolizing inducers such as phenobarbital, polychlorinated biphenyl, 3-methlcholanthrene. The rat hepatic glutathione transferase activity toward monobromobimane was elevated by about 2 to 4-folds, thus, establishing the usefulness of the assay method as a research tool for biochemical study of drug metabolism and toxicity.

Keywords: spectrofluometric, glutathione, glutathione transferase, mono-bromo-bimane.

Corresponding author

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INTRODUCTION

Glutathione-S-transferases are the most important family of enzymes involved in the metabolism of alkylating compounds and their metabolites. They are a major defence system in deactivating toxic materials (as parent compounds or metabolites) within the body [1]. It has been suggested that the action of glutathione s-transferases was to provide an activated glutathione for conjugation with any suitable electrophile [2]. Both glutathione and the second substrate are specifically bound to glutathione s-transferases [3]. Glutathione and glutathione s-transferase are located mainly in cytosol. Investigations have shown that glutathione s-transferase activity is generally greater in hepatic than extra hepatic tissues [4]. Thus, crude preparation of rat liver cytosol was used in this study.

Conjugation of glutathione with an electrophile may be followed by measurement of the amount of substrate reacted or product formed. Most of the methods utilized titrimetric [5,6] colorimetric [2] spectrophotometric [7,8,9] high performance liquid chromatography [10], or radioisotopic techniques[11].

That hepatic glutathione transferases are inducible by microsomal drug metabolizing inducers has been reported by several workers. Studies [12,13] have shown that phenobarbital was more effective inducer of rat liver ligandin than were several other substances, such as spironolactone or 3-methylcholanthrene. Phenobarbital enhanced biliary excretion of intravenously administered sulphobromophthalein on the rat was partly due to induction of glutathione s-transferase responsible for the conjugation of this compound with glutathione [14]. Kaplowizt et al [15] examined the induction of rat hepatic and renal glutathione s-transferase activity by phenobarbital, benzo[a]pyrene or 3-methylcholanthrene and reported that renal enzyme activity were induced less than those in the liver. Hepatic glutathione s-transferase activity toward styrene oxide was enhanced by 2 folds within one day of an intraperitoneal dose of a polychlorinated biphenyl mixture (100 mg/kg) to rats and remained elevated for many days [16].

Glutathione transferases catalyse glutathione conjugation with monobromobimane to yield fluorescent product which can be followed by spectrofluorimetric measurement of increase in fluorescence per minute. Thus, the aim of this study was to measure glutathione activity toward monobromobimane in induced rats by spectrofluorimetric method. The inducers were polychlorinated biphenyl, 3-methylcholanthrene and phenobarbital.

MATERIALS AND METHODS

Materials:

Four groups of Sprague-Dawley rats housed under the same conditions and fed on the same diet. The first group of rats (control) were not treated with any drug before they were

sacrificed. The second group of animals were respectively treated with one intraperitoneal injection of polychlorinated biphenyl (Aroclor 1254) 500 mg kg^{-1} and the animals were sacrificed after 7 days. The third group of rats were treated with intraperitoneal injection of 3-methylcholanthrene 20 mg $kg^{-1}day^{-1}$ for two days and the animals were sacrificed 48 hours after treatment, while the fourth group of rats were treated with 0.1% phenobarbitone in drinking water for 5 days and sacrificed 24 hours after the last treatment. All animals were sacrificed by cervical decapitation and livers collected and weighed at temperature of 0 to 4^0C. Each liver was homogenized in 3ml of 0.25 M sucrose per g of liver, and centrifuged at 3,000 and 10,000 rpm for 10 minutes respectively to precipitate cell debris. To the 10,000 rpm supernatant containing microsomes and cytosol fractions, 0.2 volume of 0.1 M calcium chloride solution was added, and further centrifuged at 10,000 rpm for 10 minutes to precipitate the microsomes. The resultant supernatant from each group of rats contained glutathione and its associate enzyme, glutathione transferase was diluted to protein concentrations in the range 2 to 4.2 mg per ml. Protein was assayed by Lowry et al method [17].

Fluorescent instrumentation	Perkin-Elmer fluorescent spectrometer model 1000 set at excitation wavelength 475nm and connected to Perkin-Elmer recorder model 56 that allowed a record of change in fluorescence with concentration.
Non-enzymic reaction mixture	3.0Mm glutathione (Sigma Chemicals), Mono-bromo-bimane in acetonitrile (1Nm) and phosphate buffer pH 6.5.
Enzymic reaction mixture	Soluble rat liver cytosol (glutathione s-transferase) containing 810µg protein/ml, 3.0 mM glutathione (Sigma Chemicals), Mono-bromo-bimane in acetonitrile and phosphate buffer pH 6.5.

For non-enzymatic reaction mixture, 100µl glutathione stock solution (to give final concentration of 100µM) and 30 µl of mono-bromo-bimane stock solution (10 µM) were pipetted into a cuvette containing phosphate buffer (3.0 ml) at pH 6.5 at 25^0C. The cuvette was inverted three times to ensure mixing and initial rate of product formation was followed by spectrofluorimetric measurement of increase in fluorescence per minute on the chart at wavelength 475 nm.

For enzymatic reaction, individual hepatic glutathione transferase solution (10-50µl) having different unknown enzyme activities were pipetted into cuvettes containing respectively glutathione solution (100µM) and phosphate buffer (3.0ml) at pH 6.5 followed by addition of monobromobimane solution (10µM). Each cuvette was immediately inverted three times to ensure mixing and the initial rate of product formation was followed by measurement of increase in fluorescence per minute.

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RESULTS

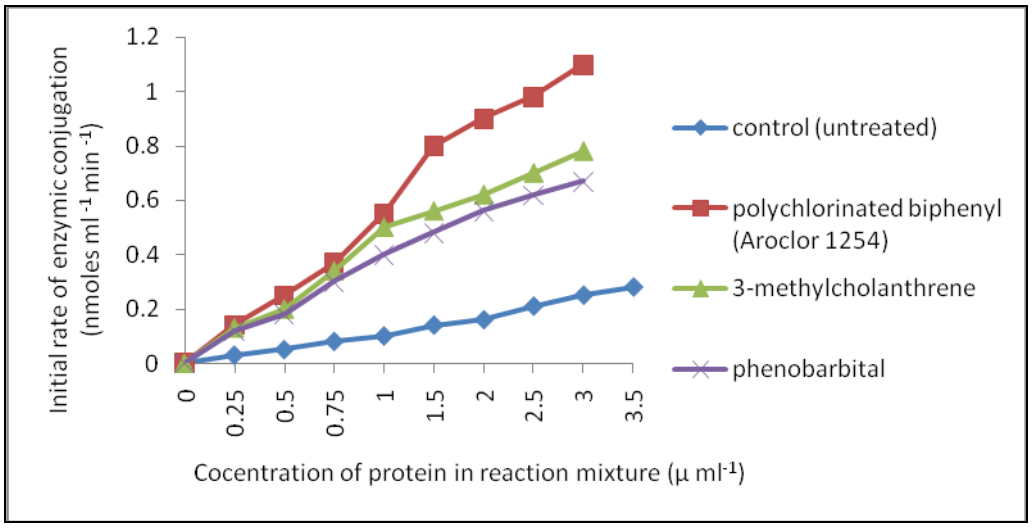

Figure 1: Effect of concentrations of rat liver cytosol preparations on the initial rate of enzymic conjugation. The cytosol prepartions were obtained from normal and induced rats respectively. Assays were conducted with monobromobimane (10 µM) and glutathione (100 µM) in phosphate buffer, pH 6.5 at 25^0C.

Figure 1 shows increased rate of enzymic conjugation with increasing concentrations of protein (from enzyme preparation) in reaction mixture. Slope of each curve gives the specific activity of the glutathione transferase in enzyme preparation under the specified common assay procedure. Table 1 shows induction of rat hepatic glutathione transferase activity toward monobromobimane after administration of enzyme inducers.

Table 1

Inducers	GSH transferase activity µmoles product/mg protein/min	Inducibility
Control (untreated)	0.15	1.0
Phenobarbital	0.42	2.8
3-Methylcholanthrene	0.52	3.4
Polychlorinated Biphenyl	0.60	4.0

The individual inducibility of glutathione transferase was expressed as the ratio of individual glutathione transferase activity toward monobromobimane in induced rat to the enzyme activity in control rat. It was found that treatment of rats with either phenobarbital (0.1% in drinking water for 7 days) or 3-methylcholanthrene resulted in about 3-fold increase in hepatic glutathione transferase activity toward monobromobimane while treatment of rats with polychlorinated biphenyl (Aroclor 1254) resulted in 4-fold increase in the enzyme activity.

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DISCUSSION

The rate of enzymatic reaction increases with increasing concentration of protein (from enzyme preparation) in presence of fixed amount of GSH and monobromobimane (Fig. 1). This indicates the assay can be used for measurement of hepatic glutathione transferase activity toward monobromobimane in normal and induced rats. Table 1 shows treatment of rat with either phenobarbitone or 3-methlychloanthrene increases the enzyme activity by 3-fold while activity in rat pre-treated with polychlorinated biphenyl (Aroclor 1254) increases by 2 to 4-folds. These observations are consistent with those reported with phenobarbital [14], 3-methylcholanthrene [13] and polychlorinated biphenyl [16] using other methods of assay.

Benson et al [20] used spectrophotometric techniques to study the elevation of extra hepatic glutathione transferase activity toward halogenated nitrobenzene by 2(3)-tert-butyl-4-hydroxy anisole (BHA). Spectroflourimetric method assay may be extended as well to examine the possibility of elevation of hepatic and extra hepatic glutathione transferase activities toward monobromobimane by BHA.

CONCLUSION

The rat hepatic glutathione transferase activity toward monobromobimane was enhanced by about 2 to 4-folds by microsomal drug metabolizing inducers consistent with those reported by several workers using other techniques of analysis, thus, establishing the usefulness of the spectroflourimetric technique of analysis as a sensitive alternative research tool for biochemical study of drug metabolism and toxicity.

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