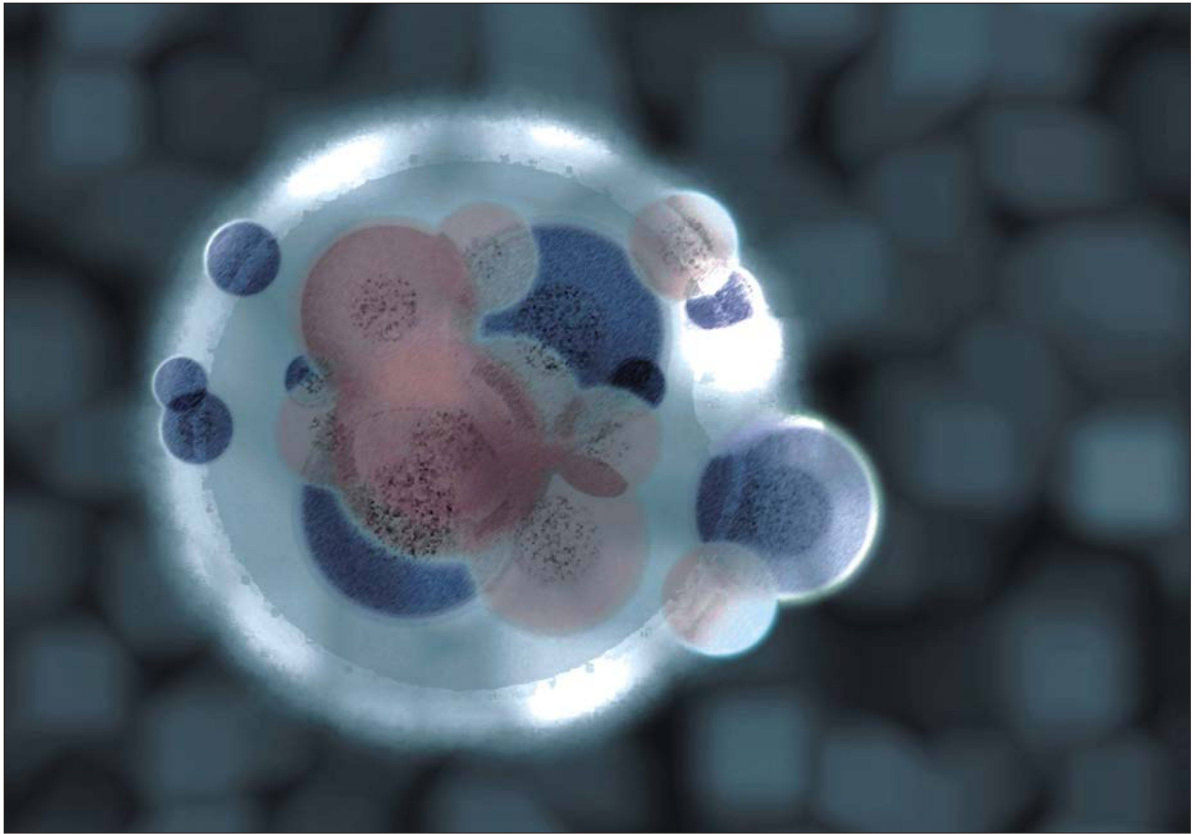

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The Distribution of Glutathione and Glutathione S-transferase Activity in the Organs of Dhub (The Agamid Lizard; *Uromastyx aegyptius*)

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Abstract: Distribution of total and reduced glutathione in addition to the glutathione S-transferase activities in Dhub (*Uromastyx aegyptius)* tissues was investigated. Studies GSH and GST are of interest because of its involvement in the detoxification and bioactivation of xenobiotics. In this study liver showed the highest level of total glutathione (6.019±1.38 μmol g^{-1} wet tissue), whereas, the heart has the lowest level (1.753±0.255 μmol g^{-1} wet tissue). Reduced glutathione (GSH) was three times higher in the liver than in the heart. The distribution of glutathione S-transferase activity was examined in the liver, kidney, lung and heart. The bulk of glutathione S-transferase activity was found in the liver (3.75 μmol min^{-1} mg^{-1} protein) and the lowest in the heart (0.27 μmol min^{-1} mg^{-1} protein).

Key words: GST, GSSG, CDNB, tissue distribution

INTRODUCTION

Dhub is a member of the old world lizard family: Agamidae. Dhub population is distributed throughout Arabia, southern Iraq, Jordan and Syria. An adult Dhub may weigh up to 2 kg. This reptile is a diurnal one and becomes active during the warm season in temperatures ranging from 39 to 41°C (Varnet *et al*., 1988). It hibernates during winter in its burrows for a period of 2 to 5 months. Dhub feeds on a large variety of plant species as well as on some insects such as grasshoppers and beetles. The Dhub (*Uromastyx aegyptius*) is a solid yellowish color with a usual size of 40 cm of which almost half of this consists of the tail. Males are a little larger than females but generally it is best to differentiate between male and female by the presence of prefemoral pores in males. These organisms needs strong detoxification ability to face pollutants in their habitat.

Among the different detoxifying enzymes, glutathione S-transferase (GST) [EC: 2.5.1.18] play a major role. GSTs constitute a multifunctional family of mainly cytosolic biotransformation enzymes involved in protecting tissues from toxins (Khurana *et al*., 2002). These enzymes catalyze the conjugation of intracellular glutathione (GSH, δL-glutamyl-L-cysteinyl glycine) to a wide variety of chemicals possing electrophilic centers, and the final GSH-conjugates have increased hydrophilicity, which facilitates their further metabolism and elimination. Glutathione S-transferase is a widely distributed enzyme present in hepatic and extrahepatic tissues of vertebrate species including humans (Simons and Vander-Jaget, 1977; Awasthi *et al*., 1994; Thomson *et al*., 2004), rats (Habig *et al*., 1974; Thomson *et al*., 2004), sheep (Reddy *et al*., 1983), rabbit and camel (Hunaiti and Abu Khalaf, 1986; Hunaiti and Sarhan, 1987; Hunaiti *et al*., 1988; Hunaiti and Asa'd, 1989; Raza and Montague, 1993; Raza *et al*., 1997).

Glutathione is one of the most naturally occurring tripeptide isolated from animal and plant cells. This intracellular non-protein thiol compound is a constituent of all eukaryotic cells and is involved in different types of chemical reactions in the biological system. Besides maintaining cellular integrity by creating a reduced environment, glutathione has multiple functions including detoxification of xenobiotics and participation in the synthesis of proteins and nucleic acids (Gerard-Monnier and Chaudiere, 1996; Rahman *et al*., 1999; Dickinson and Forman, 2002). The effectiveness of glutathione protection of tissues depends largely on several factors: (i) concentration of glutathione in the tissue; (ii) ability of the tissue to import reduced (GSH) and (iii) to export oxidized glutathione (GSSG). Several studies have reported that the concentration of glutathione is high in the liver and is found mainly in a reduced form (Chasseaud, 1979; Sies and Akerboom, 1983; Meister, 1995). GSH peroxidasee converts GSH to GSSG which can be converted back to GSH by GSH reductase (Akerboom and Sies, 1981). To our knowledge, the

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biochemical processes such as glutathione metabolism in this reptile receives no interest. Therefore, the present study investigates the distribution of glutathione and glutathione S-transferase activity in the selected organs, because of the lack of biochemical knowledge concerning the detoxification system in the Dhub.

MATERIALS AND METHODS

Glutathione reductase (EC 1.6.4.2) C type III (150-units mg^{-1} protein), Glyoxalase I (EC 4.4.1.5) grade IV (700 units mg^{-1} protein), reduced glutathione (GSH), oxidized glutathione (GSSG), Nicotinamide adenine dinucleotide phosphate-reduced form (NADPH), 5,5`-dithiobis (2-nitrobenzoic acid) (DTNB), methyl glyoxal sodium deoxycholate, bovine serum albumin s(BSA) were purchased from Sigma (USA). 1-chloro-2, 4-dinitrobenzene (CDNB) from Aldrich (USA). All chemicals are of the highest quality reagents.

Animals: Three male Dhub (*Uromastyx aegyptius*) inhabits dry habitats were captured from areas around Riyadh region (Saudi Arabia) in late August.

Tissue preparation: Fresh Dhub liver samples were rapidly weighed (an average of 32 g) were cut into small pieces and homogenized using an electrical homogenizer (Edmund Buhler 7400, Germany) operating at maximum speed for 1 min in a buffer containing 0.1 M phosphate buffer (pH 6.5) in a ratio of 1:3 (w/v). Lung samples (an average of 22 g) were homogenizaed as described above. Kidney (4 g) and heart (5.5 g) were separately homogenized using a Potter-Elvehjem homogenizer in a buffer containing 0.1 M phosphate buffer (pH 6.5) in a ratio of 1:2 (w/v), respectively. All homogenates were centrifuged at 12,000 x g for 45 min. The supernatant fractions were separated and filtered through glass wool to remove floating lipids. Except the cellular glutathione levels, which was performed with unfrozen samples (4°C), the glutathione S-transferase assay was performed with homogenates stored at -70°C. This step is important to prevent protease action on glutathione S-transferase (GST).

The experimental study was performed on three homogenates from three male Dhub and triplicate analysis from each homogenate was performed.

Measurement of total glutathione in tissue extract: Biological samples containing glutathione (GSH) and glutathione disulfide (GSSG) were deproteinized by metaphosphoric acid. Total glutathione content was determined spectrophotometrically according to the method modified by Floreani *et al.* (1997). Briefly, Supernatants (0.1 mL) obtained from different organs were diluted 1:5 by the addition of 0.4 mL of 0.1 M potassium phosphate (pH 7.4). Subsequently, 0.3 mL of diluted supernatant was transferred to the reaction mixture that contains 0.1 M potassium phosphate; 5 mM EDTA; pH 7.4; 0.25 mM, 5,5'-dithiobis (2-nitrobenzoic acid) 5,5`-dithiobis-2-nitrobenzoic acid (DTNB, prepared in 0.1 M potassium phosphate, pH 7.4) and 0.4 mM NADPH in a final volume of 3 mL. The mixture was incubated at 25°C for 3 min and the reaction was started by the addition of 2U of glutathione reductase (diluted in 0.1 M potassium phosphate, EDTA, pH 7.4). The formation of TNB was continuously recorded at 412 nm at 25°C.

The total amount of glutathione of an unknown (sample) was determined by calculating from the linear equation generated from several standards of glutathione. GSH standards are prepared daily in 0.1 M potassium phosphate, 5 mM EDTA, pH 7.4.

Measurements of reduced glutathione in tissue extract: Reduced glutathione in tissue extracts was determined spectrophotometrically using the method of Akerboom and Sies (1981). The reaction was carried out in a 3 mL reactions containing 0.05 M potassium phosphate, (pH 7.0), 60 μL of sample, 6 μL glyoxalase I (1000 units mL^{-1}). The reaction was started by the addition of 60 μL of 110 mM methylglyoxal and the enzymatic formation of s-lactoyl-glutathione was continuously recorded at 240 nm.

GSSG concentration in samples is calculated as the difference between total glutathione and reduced glutathione.

CDNB- GST assay: CDNB-GST assays were conducted according to the method described by Habig *et al.* (1974) in 3 cm cuvettes, the reaction mixture consisted of 0.1 M potassium phosphate, pH 6.5, 1.6 mM GSH and 1 mM CDNB. The reaction was initiated with homogenate addition, mixing prior to recording absorbance changes. CDNB-GSH conjugation (formation of dinitrophenyl thioether-glutathione conjugation [DNP-SG] vianucleophilic displacement of CL with the GSH thiol) was monitored spectrophotometrically at $\lambda = 340$ nm for 2 min. DNP-SG concentration was calculated with an extinction coefficient of 9.6 mM^{-1} cm^{-1} (Habig *et al.*, 1974). Enzyme preparations for each tissue were assayed in triplicate. Control reactions (with complete assay mixture without the enzyme) were included to determine nonenzymatic CDNB-GSH conjugation.

Determination of total protein: Protein content of each sample was determined spectrophotometrically at 750 nm according to the method described by Lowry *et al.* (1951) using bovine serum albumin as slandered.

RESULTS

The level of glutathione, one of the major constituents of eukaryotic cells and is involved in detoxification of toxic chemicals that may exist in cells, was determined in Dhub organs. This study reveals that the liver exhibited the highest level of total glutathione (GSH and GSSG) measuring 6.019±1.38 μmol g^{-1} wet tissue. However, there is also significantly high levels of this tripeptide in certain extrahepatic tissues, most noticeably in the kidney measuring 3.528±0.45 μmol g^{-1} wet and in the lung measuring 1.964±0.017 μmol g^{-1} wet tissue. The lowest amount, however, was found in the heart measuring only 1.753±0.255 μmol g^{-1} wet tissue, (Table 1).

GSH content in the Dhub liver was (5.959±1.36 μmol g^{-1} wet tissue), while in kidneys, lungs and heart the levels were 3.493±0.453, 1.944±0.017 and 1.735±0.253, respectively (Table 2).

Liver exhibited the highest activity of GST (3.75 μmol min^{-1} mg^{-1} protein), whereas, the lowest activity was found in the heart (0.27 μmol min^{-1} mg^{-1} protein). The activity of GST in kidneys and lungs was 1.43 and 0.46 μmol min^{-1} mg^{-1} protein, respectively (Table 3).

Table 1: Total Glutathione contents of various tissues

Tissue	Wet tissue (μmol g^{-1})	Liver (%)
Liver	6.019±1.38	100.0
Kidney	3.528±0.45	58.4
Lung	1.964±0.017	32.5
Heart	1.753±0.255	29.0

Each value is the mean±SD from 3 determinations carried out on duplicate

Table 2: Reduced Glutathione contents of various tissues

Tissue	Wet tissue (μmol g^{-1})	Liver (%)
Liver	5.959±1.36	100.0
Kidney	3.493±0.453	58.7
Lung	1.944±0.017	32.5
Heart	1.735±0.253	29.2

Cyosolic GSH concentration (μmol g^{-1} wet tissue) was measured as protein free-SH content using dithiobisnitrobenzoic acid. Values are mean±SD from three determinations in triplicates

Table 3: Cytosolic Glutathione S-transferase activity of various tissues

Tissue	Specific activity	Liver (%)
Liver	3.75	100.0
Kidney	1.43	38.1
Lung	0.46	12.3
Heart	0.27	7.2

Values are expressed as μmol min^{-1} mg^{-1} protein

DISCUSSION

Biotransformation of chemicals by the addition of glutathione, a reaction catalyzed by GST, is one of the most versatile protective mechanisms in eukaryotic cells. The enhanced water solubility of compounds, after the addition of glutathione, facilitates their excretion from cellsv and thereby prevents their accumulation in the body.

This study shows that various organs of the Dhub possess variable amounts of GSH, GSSG and GST activity, which reflect one of the major pathways of detoxification of xenobiotics. Although most tissues possess the capacity to synthesize GSH from its amino acid precursors, the major organ releasing GSH is the liver (Akerboom and Sies, 1981; Akerboom *et al.*,1997). Results presented in this study are in total agreement with other studies that show that the highest content of GSH in animals is in the liver (Chasseaud, 1979; Sies and Akerboom, 1983; Meister, 1995). This confirms the fact that the liver is the major organ responsible for the detoxification of various exogenous and endogenous toxicants in mammals (Chasseaud, 1979) and reptiles (this study). Likewise, kidney and lung are exposed to toxicants and therefore, must possess their own GSH and GST activity. Such compounds may play an essential role in protecting these tissues from the harmful effects of toxic compounds. The relatively low levels of GSH and GST in the heart reported in this study are probably more than adequate to detoxify endogenous and exogenous toxicants that might be present in the heart.

The activity of GST in the liver, kidneys, lungs and heart changes in a pattern similar to the changes in the GSH level. The fact that GSH level and GST activities are lower in the lung and heart than in liver and kidney may render these organs more susceptible to the effect of toxicants.

In summary, the variables analyzed in this study were found to be highest in the liver of Dhub. This suggests that this tissue had the highest antioxidant enzyme activity to counteract the oxidative damage.

REFERENCES

Akerboom, T.P.M. and H. Sies, 1981. Transport of Glutathione Disulfide and Glutathione Conjugates Across the Hepatocyte Plasma Membrane. New York: Academic Press, pp: 245-251, 523-534.

Akerboom, T.P.M., Y.Ji., G. Wagne and H. Sies, 1997. Subunit specificity and organ distribution of glutathione transferase-catalysed S-nitrosoglutathione formation from alkyl nitrites in the rat. Biochem. Pharmacol., 53: 117-120.

Awasthi, Y.C., R. Sharma and S.S. Singhal, 1994. Human glutathione S-transferase. Int. J. Biochem., 26: 295-308.

Chasseaud, L.F., 1979. The role of glutathione and glutathione S-transferase in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res., 29: 175-274.

Dickinson, D.A. and H.J. Forman, 2002. Cellular glutathione and thiole metabolism. Biochem. Pharmacol., 64: 1019-1026.

Floreani, M., M. Petrone, P. Debetto and P. Palatini, 1997. A comparison between different methods for the determination of reduced and oxidized glutathione in mammalian tissues. Free Radic Res., 26: 449-455.

Gerard-Monnier, D. and J. Chaudiere, 1996. Metabolism and antioxidation function of glutathione. Pathol. Biol., 44: 77-85.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Hunaiti, A.A. and I.K. Abu Khalaf, 1986. The distribution and comparison of glutathione, glutathione reductase and glutathione S-transferase in various camel tissues. Comp. Biochem. Physiol., B 85: 733-737.

Hunaiti, A.A. and M.A. Sarhan, 1987. Purification and characterization of camel liver glutathione S-transferase. Int. J. Biochem., 19: 71-77.

Hunaiti, A.A., S.T. Abu-Orabi, M.A. Sahran and W.M. Owais, 1988. Interaction of organic azides with purified camel glutathione S-transferase. Biochem. Med. Metab. Biol., 39: 140-147.

Hunaiti, A.A. and Q. Asa'd, 1989. Camel brain glutathione S-transferase purification, properties, regional and subcellular distribution. Comp. Biochem. Physiol., B 93: 333-338.

Khurana, S., M.T. Corbally, F. Manning, T. Armenise, B. Kierce and C. Kilty, 2002. Glutathione S-transferase: A potential new marker of intestinal ischemia. J. Pediatr. Surg., 37: 1543-1548.

Lowry, O.H., NJ. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Biochem., 193: 265-275.

Meister, A., 1995. Glutathione metabolism. Methods Enzymol., 251: 3-7.

Rahman, Q., P. Abidi, F. Afaq, D. Schiffmann, B.T. Mossman, D.W. Kamp and M. Athar, 1999. Glutathione redox system in oxidative lung injury. Crit. Rev. Toxicol., 29: 543-568.

Raza, H. and W. Montague, 1993. Drug and xenobiotic metabolising enzymes in camel liver: Multiple forms and species specific expression. Comp. Biochem. Physiol., C, 104: 137-145.

Raza, H., M.S. Lakhani, I. Ahmed, A. John, R. Morgenstern and W. Montague, 1997. Tissue specific expression of glutathione s-tansferases, glutathione content and lipid peroxidation in camel Tissues. Comp. Biochem. Physiol., B 118: 829-835.

Reddy, C.C., J.R. Burgess, Z.Z. Gong, E.J. Massaro and C.P. Tu, 1983. Purification and characterization of the individual glutathione S-transferase from sheep liver. Arch. Biochem. Biophys., 224: 87-101.

Sies, H.B.R. and T.P.M. Akerboom, 1983. Intrahepatic Glutathione Status. In: Function of Glutathione Biochemical Physiological Toxicological and Clinical Aspects. Al, Le. (Ed.), New York: Raven Press., pp: 51-64.

Simons, P.C. and D.L. Vander-Jaget, 1977. Purification of glutathione S-transferase from human liver by glutathione affinity chromatography. Ann. Biochem., 82: 334-340.

Thomson, R.E., A.L. Bigley, J.R. Foster, I.R. Jowsey, C.R. Elcombe, T.C. Orton and J.D. Hayes, 2004. Tissue-specific expression and subcellular distribution of murine glutathione S-transferase class kappa. J. Histochem. Cytochem., 52: 653-662.

Varnet, R., M. Lemire, C.J. Grenot and J. Francaz, 1988. Ecophysiological comparisons between large Saharan lizards *Uromastyx Acanthinurus* (agamidae) and *Varanus Griseus* (Varamidae). J. Arid. Environ., 14: 187-200.