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Glutathione Depletion by Xenobiotics in *Coturnix coturnix japonica*

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Toxicological research on birds can be applicable to human health both directly by their use as models for mechanistic and descriptive studies and indirectly as monitors of environmental quality. The biochemical and physiological responses of birds to chemical insult are frequently used as biomarkers to evaluate pollution hazards (Fossi et al. 1986; Fox et al. 1988; Massi et al. 1991). It is very difficult to extrapolate biological effects between species because of their different distribution of chemicals, metabolism, detoxification and excretion (Van Straalen 1994). However It has been observed that differences in the metabolism of xenobiotics between birds and mammals are more quantitative than qualitative. Differences were found in the N-acetylation of sulphanilamide, the aromatic hydroxylation of aniline and the benzoic acid conjugation with glycine and glucuronic acid (Pan and Fouts 1979), while inducers of cytochrome P450 increase several hepatic cytochrome P450-mediated activities both in avian and mammalian liver (Ronis and Walker 1989).

Although many studies on the mixed-function oxygenase (MFO) system and its induction have been carried out in domestic and wild birds, the investigations on glutathione in birds are limited. The biological functions of glutathione in detoxification of toxic compounds, metals, oxygen and radiation have been well documented in mammalian systems (Meister and Anderson 1983; Taniguchi et al. 1989). However, comparatively little is known about glutathione in avian species. In view of the hypothetical use of glutathione in avian species as a biomarker of exposure to pollutants, we performed a preliminary study on Japanese quail (*Coturnix coturnix japonica*), evaluating the effect of some glutathione-depleting agents.

MATERIALS AND METHODS

Acetaminophen (AA), diethylmaleate (DEM), DL-buthionine-[SRI-sulfoximine (BSO) and L-methionine sulfoximine (MSO) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Adult female Japanese quails weighting 130-150 g were obtained from a local supplier. They were housed

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under controlled environmental conditions of temperature (22 ± 1°C) and light (12 h light-12 h dark) and had free access to water and bird food. For experimentation the quails were randomly divided into groups of 5 animals each, and the tested compounds were administered intraperitoneally.

AA dissolved in warm 0.9% saline solution was administered at doses ranging from 80 to 750 mg/kg. BSO and MSO dissolved in 0.9% saline solution were injected at a dose of 2.5 and 3 mmoles/kg. DEM in corn oil was administered at doses ranging from 0.3 to 1.5 mL/kg. Control animals received vehicle only (saline or corn oil). After the appropriate time interval (15, 30, 60, 120, or 240 min) animals were sacrificed, whole blood was drawn into heparinized tubes and specimens of liver, kidneys, and brain were collected. Tissues were excised and immediately rinsed in ice-cold 0.9% (w/v) NaCl solution. The chilled tissues were trimmed, weighed, and quickly stored at -80°C until glutathione assay. A 0.2 ml portion of blood was added to 0.8 mL of ice-cold 5% (w/v) metaphosphoric acid, incubated at room temperature for 15 min, and centrifuged at 14,000 x g for 10 min. The supcrnatant obtained was immediately frozen and stored at -80°C until glutathione assay.

Levels of reduced (GSH) and oxidized (GSSG) glutathione were determined using *o*-Phthalaldehyde (OPT) as fluorescent reagent according to the method of Hissin and Hilf (1976). The frozen tissues were homogenized in 3 volumes of 1 M sodium phosphate-5 mM EDTA buffer pH 8, then 1 volume of ice-cold 25% (w/v) metaphosphoric acid was added. Homogenates were centrifuged at 105,000 x g for 30 min to precipitate the protein. The reaction mixture contained 1.8 mL of phosphate-EDTA buffer, 100 µL of diluted supcrnatant and 100 µL of OPT solution containing 100 µg of OPT. After 15 min, the intensity of fluorescence at 420 nm wavelength was determined with excitation at 350 nm. For GSSG assay a portion of the supernatant was incubated at room temperature with 0.04 M N-ethylmaleimide (NEM). The reaction was stopped after 30 min by the addition of cold 0.1 N NaOH. This mixture was taken for measurement of GSSG using the procedure for GSH assay, except that 0.1 N NaOH was employed as diluent rather than phosphate-EDTA buffer.

Data in the text (expressed as mean ± SEM) were estimated by two way analysis of variance (ANOVA) followed by the Newman-Keuls test. The Student's t test was also used when comparing the mean values from the treated quail with those from the control. The difference was considered significant if the corresponding p value was less than 0.05.

RESULTS AND DISCUSSION

The administration of AA (750 mg/kg) to quail led to a time-dependent loss of both reduced and oxidized glutathione (Fig. 1). There were no consistent

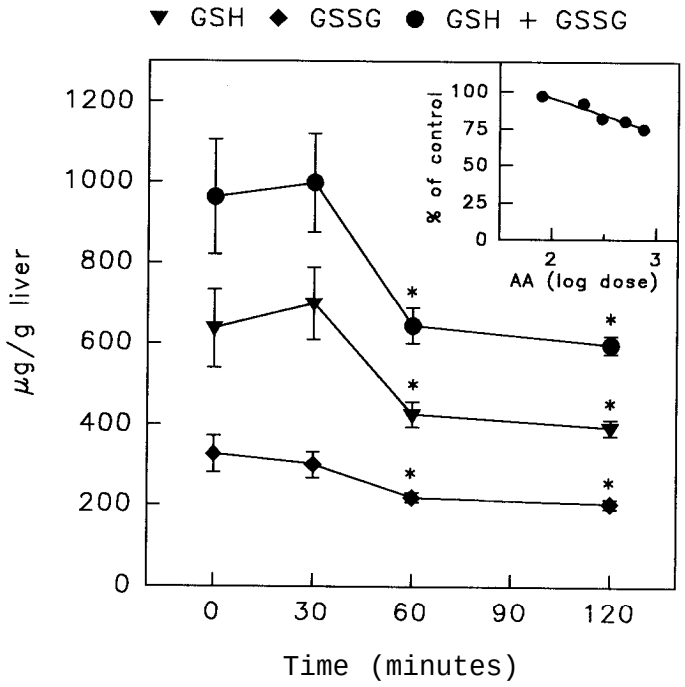

Figure 1: Time-dependent depletion of hepatic glutathione induced by AA (750 mg/kg) in quail. * $p<0.05$ vs. control (time 0) (ANOVA followed by Newman-Keuls test). (*Inset*) Effect of AA at doses ranging from 80 to 750 mg/kg on total glutathione after 2 hr.

differences in the amount of glutathione when control and AA-treated quail were compared at 30 min. Glutathione levels were significantly depressed by 1 hr postinjection in quail treated with AA. Depletion of both reduced and oxidized glutathione was about 35% in treated birds and these differences were maintained for 2 hr. The depletion induced by AA was also dose-dependent, where birds treated at doses from 80 to 750 mg/kg after 2 hr exhibited a significant decrease in the levels of hepatic glutathione ($r=0.9697$, $p<0.05$) (Fig. 1 *Inset*) .

As observed in mice (Mitchell et al. 1973), administration of DEM to quail caused a greater decline in hepatic glutathione than those treated with AA (Fig. 2). The kinetics of depletion induced by DEM at a dose of 1 mL/kg shows that reduced glutathione was significantly decreased 15 min after injection. The greatest effect (55% of depletion) was found 30 min after DEM administration and the values remained at these levels for 2 hr. Although oxidized glutathione levels were slightly enhanced 15 min after DEM injection, they were already decreased 1 and 2 hr after treatment.

Depletion of total glutathione induced by DEM at doses ranging from 0.3 to 1.5 mL/kg was dose-dependent (Fig. 2 *Inset*). There was a marked and highly

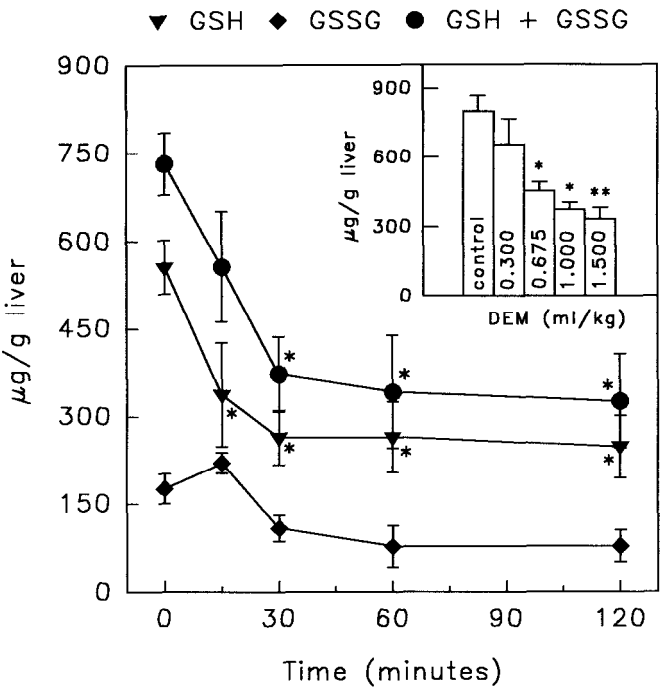

Figure 2: Time-dependent depletion of hepatic glutathione induced by DEM (1 mL/kg) in quail. (*Inset*) Effect of DEM at doses ranging from 0.3 to 1.5 mL/kg on total glutathione after 2 hr. * $p<0.05$, ** $p<0.01$ vs. control (ANOVA followed by Newman-Keuls test).

significant glutathione decrease (approx. 60%) in quail treated with DEM at a dose of 1.5 mL/kg. The depletion in the level of glutathione was also significant at lower doses of treatment.

BSO which in mammals inhibits the activity of γ-glutamylcysteine synthetase and, therefore, the synthesis of glutathione. The administration of BSO at a dose of 3 mmoles/kg led to a time-dependent loss of both reduced and oxidized glutathione in quail (Fig. 3). In particular a marked and significant decrease in the level of reduced glutathione was observed in the liver after 1 hr and a further depletion occurred after 2 and 4 hr. Although the depletion of oxidized glutathione appeared noticeable (40% after 2 hr), the decrease was not statistically significant. The hepatic glutathione in quail was also significantly depleted by administration of MSO, an inhibitor of glutamine synthetase (Fig. 3 *Inset*). The decrease in the content of total glutathione induced by MSO (50% after 2 hr) was higher than that caused by BSO (40% after 2 hr), suggesting that quails are more sensitive to methionine analogue. Moreover, in all birds, treatment with MSO produced convulsions within 90 min and this effect was observed at doses lower than those required to induce convulsions in rats (Palekar et al. 1975).

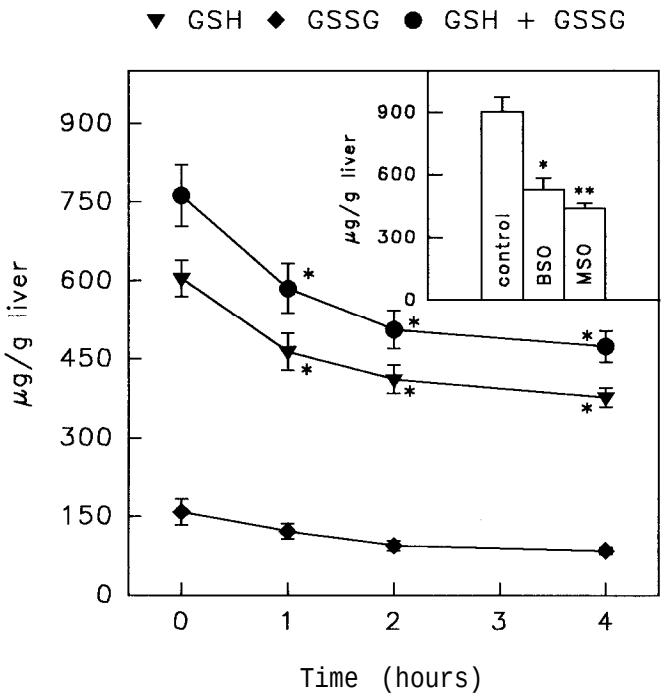

Figure 3: Time-dependent depletion of hepatic glutathione induced by BSO (3 mmoles/kg) in quail. (*Inset*) Effect of BSO and MSO (2.5 mmoles/kg) on total glutathione after 2 hr. * $p<0.05$, ** $p<0.01$ vs. control (ANOVA followed by Newman-Keuls test).

Additional experiments were performed to determine the effect of glutathione-depleting agents in other tissues and biological samples. In particular we have investigated the effect of DEM and BSO on blood glutathione because it may be collected by a "non-invasive" method, without killing the birds.

The results show that administration of DEM (1 mL/kg) to quail induced a significant decrease in reduced glutathione in all organs (Fig. 4). The depletion in the liver, kidney, and brain after 2 hr amounted to 55, 34, and 40% respectively. A similar effect was observed in liver and kidney after administration of BSO (3 mmoles/kg); no differences were found in the levels of brain glutathione even 4 hr (data not shown). This indicates that for quail, as in rats, the rate of synthesis of brain glutathione is relatively slow when compared to liver and kidney.

Significant depletions in the content of glutathione were also observed in whole blood, where the depleting effect of DEM was more evident than for BSO. The administration of BSO caused a 30% decrease in blood glutathione compared to a 85% depletion was induced by DEM.

The results presented here indicated that the basal content of glutathione and the glutathione depletion in quail are similar to those observed in mammals.

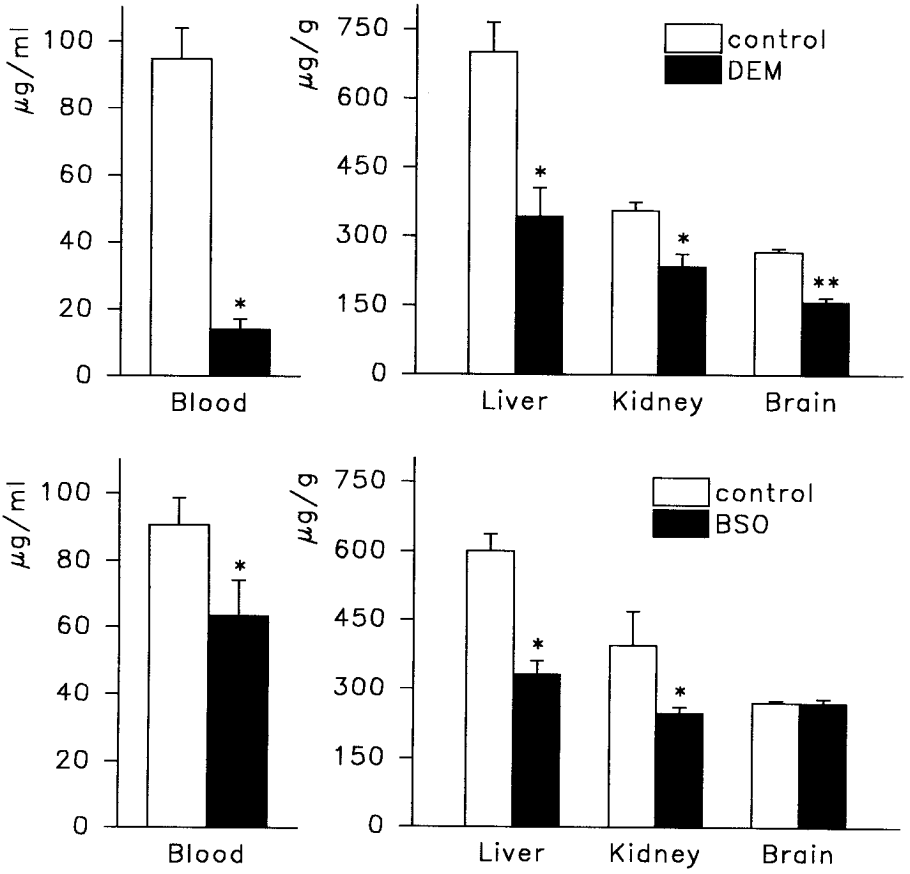

Figure 4: Effect of DEM (1 mL/kg) and BSO (3 mmoles/kg) on blood, liver, kidney, and brain of quail. Levels of reduced glutathione were determined after 2 hr. * $p<0.05$, ** $p<0.01$ vs. control (Student's *t* test).

Differences are mostly quantitative and not qualitative, suggesting that this alternative animal model can be useful for toxicological studies concerning oxidative stress and as a monitor of environmental quality.

In ecotoxicological studies, biomarker is commonly defined as "molecular, biochemical and cellular change caused by pollutant chemicals which is measurable in cells, tissues and body fluids" (McCarthy and Shugart 1990). Our observation that levels of glutathione in quail decreased after administration of glutathione-depleting agents adds further support to the idea that avian species could be used as biomonitors. The results also showed the reliability of the measurement of glutathione in whole blood by a "non-invasive" method.

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