Kinetics and Dose Dependence of Glutathione, Glutathione-S-Transferase and Phosphoglucomutase in Liver and Kidney of Nickel Treated Partially Hepatectomized Rats

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Abstract: Administration of nickel (50 μ moles Ni/kg as NiCl₂·6H₂O, subcutaneously) to partially hepatectomized rats at 8, 16, 24 and 72 hrs revealed that it did not produce any change in the levels of hepatic or renal glutathione at any time interval when compared with saline treated hepatectomized rats. Remarkable changes were however observed in glutathione-S-transferase and phosphoglucomutase at 16 and 24 hrs in liver while only at 8 hrs in kidney. The effect of various doses of nickel (50, 100 and 150 μ mole Ni/kg) at 16 hrs revealed that 100 μ moles nickel produced maximum enhancement in the levels of all these parameters in liver while in kidney practically no change was observed.

Key words: Nickel intoxication—Glutathione—Glutathione-S-transferase—Phosphoglucomutase—Partially Hepatectomized rats

INTRODUCTION

The kinetics and metabolism of nickel have received attention due to its toxic potential and undetermined role as an essential trace element in human and animals.1-5) Nickel like other heavy metals is known to bind with sulfhydryl moiety in biological system as a part of its detoxication process.5-7) Glutathione, a sulfhydryl tripeptide plays an important role in maintaining the sulfhydryl enzymes and the integrity of the cells.7,8) It also acts as a γ-glutamyl donor in the γ-glutamyl cycle and maintains the cysteine pool in the system.9) Glutathione-S-transferase is an important enzyme which plays crucial role during the metabolic disposition of a variety of xenobiotics through conjugation with glutathione.10-12) Phosphoglucomutase another sulfhydryl dependent enzyme is responsible for the turnover of triphosphopyridine nucleotide (NADPH) via

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The phosphoglucone pathway, the latter plays an important role as a cofactor as well as maintains the redox potential of the system.\textsuperscript{13,14} The extent of toxic manifestations is known to be affected by several factors including age. The growing subjects are reported to be particularly susceptible to all forms of potentially toxic chemicals.\textsuperscript{15,16} Regenerating rat liver after two-thirds hepatectomy serves as an excellent model for studying the effect of toxic substances on the growing system. Since glutathione plays an important role in the detoxication process in the absence of efficient drug metabolizing enzymes in growing systems,\textsuperscript{17} the present investigations have been undertaken to study the effect of nickel on glutathione, glutathione-S-transferase and phosphoglucomutase in liver and kidney of partially hepatectomized rats at various time intervals after its administration. The dose dependent effect of nickel on these parameters have also been examined.

**Materials and Methods**

Male albino rats (78 Nos.) of Industrial Toxicology Research Centre colony weighing 125–150 gm were used in the present study. A group of 6 animals were killed by cervical dislocation and served as normal controls for successive experiments. The rest 72 animals were subjected to partial hepatectomy under mild ether anaesthesia.\textsuperscript{18} These hepatectomized rats were divided into two experimental groups of 48 and 24 animals each. Twenty-four animals in the first experimental group received single subcutaneous (sc) injection of nickel (50 μ mole Ni/kg/ml as NiCl\(_2\cdot6\)H\(_2\)O (Fluka, Switzerland) in 0.9% NaCl just after partial hepatectomy while the other 24 animals received an equivalent volume of 0.9% NaCl only and served as control. Six animals each from both the groups were killed at 8, 16, 24 and 72 hrs after nickel or 0.9% NaCl administration.

In the second experiment, 24 partially hepatectomized rats were divided into four groups of six animals and received 0.9% NaCl, 50 μ mole Ni, 100 μ mole Ni or 150 μ mole Ni/kg respectively through the same route. All the animals were killed by cervical dislocation at 16 hrs following treatment.

Liver and kidney were immediately removed, washed free of extraneous material and homogenized in ice cold 0.25 M sucrose in a Potter Elvehjem homogenizer. Glutathione content was determined in whole homogenate by the modified method of Jollow et al.\textsuperscript{19} Equal volumes of tissue homogenate (10%) and sulfosalicylic acid (4%) were mixed and kept in cold for 1 hr. It was centrifuged at 3000 rpm at 4\(^\circ\) for 10 min. An aliquot (0.2 ml) of supernatant was mixed to phosphate buffer (0.1 M, pH 7.4, 4.0 ml) and 5,5-dithiobis-2-nitrobenzoic acid (DTNB 0.1 M, pH 7.4, 0.2 ml). The resulting solution was kept at room temperature for 30 min, and absorbance read at 412 nm against blank containing buffer and DTNB.
The remaining homogenate was further subjected to centrifugation at 9000 × g for 20 min at 4°C to obtain post mitochondrial supernatant for the assay of glutathione-S-transferase and phosphoglucomutase activity. The activity of glutathione-S-transferase was measured by the method of Habig et al.\textsuperscript{20}) The assay mixture contained phosphate buffer (0.1 M, pH 6.5, 2.9 ml), glutathione (1.0 mM, 0.05 ml), 1-chloro-2, 4-dinitro-benzene (CDNB) (1.0 mM, 0.02 ml) and enzyme (post mitochondrial supernatant of 5% homogenate, 0.1 ml). Change in absorbance was noted at every 15 seconds for 2 min at 340 nm against the suitable blank.

The activity of phosphoglucomutase was determined by the procedure of Najjar.\textsuperscript{21}) The reaction mixture contained magnesium sulfate (0.006 M, 0.1 ml), glucose-1-phosphate (0.02 M, pH 7.5, 0.1 ml), cystein- HCl (0.1 M, pH 7.5, 0.1 ml), enzyme (post mitochondrial supernatant of 5% homogenate, 0.1 ml) and water to make the volume 1.5 ml. The mixture was incubated for 5 min at 30°C. The reaction was stopped by adding 1 ml of 5N H$_2$SO$_4$. After adding 2.5 ml water the tubes were kept on boiling water bath for 3 min cooled and centrifuged for 10 min. One ml aliquot of supernatant was used for determination of inorganic phosphorus (Pi).

**RESULTS**

*Effect of partial hepatectomy in rats:*

The alterations in hepatic and renal glutathione, glutathione-S-transferase and phosphoglucomutase at various time intervals after partial hepatectomy are
shown in Figs. 1–3. An increase in glutathione level was observed only in liver at 24 hrs which returned to the normal control value by 72 hrs. Glutathione-S-transferase activity was considerably decreased at 16 hrs in liver and was restored at 24 hrs while in kidney an induction was observed at 24 hrs which was maintained even upto 72 hrs. The activity of hepatic phosphoglucomutase was
inhibited at 24 hrs which returned to the normal control value by 72 hrs while the
renal activity showed inhibition at 8 hrs which was restored at 16 hrs.

**Effect of nickel in partially hepatectomized rats:**

The effects of nickel administration (50 μ mole/kg sc.) on the hepatic and
renal levels of the three parameters in partially hepatectomized rats at various

**Fig. 4.** Effect of various doses of nickel on glutathione levels in liver and
kidney at 16 hrs after its administration in partially hepatectomized
rats.

**Fig. 5.** Effect of various doses of nickel on glutathione-S-transferase activity
in liver and kidney at 16 hrs after its administration in partially hepatectomized_rats.
time intervals are also shown in Figs. 1–3. Nickel did not produce any change in the levels of either hepatic or renal glutathione at any time interval. The activity of hepatic glutathione-S-transferase was significantly induced at 8, 16 and 24 hrs which returned to the normal control value by 72 hrs. Its activity was also induced in kidney but only at 8 hrs. The pattern of phosphoglucomutase activity was found to be similar to that of glutathione-S-transferase both in liver and kidney.

The effect of different doses of nickel (50, 100 or 150 μmole/kg) on these parameters at 16 hrs in partially hepatectomized rats are shown in Figs. 4–6. The dose dependent study revealed an induction in the level of glutathione in liver by 100 and 150 μmole nickel. While in kidney no change was observed at any of the administered doses. The activity of glutathione-S-transferase and phosphoglucomutase was significantly enhanced in liver at all the administered doses when compared to normal values, while they remained unaffected in kidney.

**DISCUSSION**

Partial hepatectomy is reported to reduce the levels of drug metabolizing enzymes and to enhance the cellular degradation of heme. Thus the ability of cells to detoxify xenobiotics is considerably diminished. The enhanced level of hepatic glutathione in partially hepatectomized rats may thus provide a protective mechanism for the detoxication process although contributions from other biotransforming mechanisms cannot be excluded. Recent studies from our laboratory also indicate that the enhancement in glutathione is also accompanied
by the increased activity of glutathione reductase, the latter being responsible to maintain the level of glutathione.\textsuperscript{17)

The impaired level of both hepatic and renal phosphoglucomutase also suggests the imbalance in the redox potential of the system which might be responsible for changes in the activity of drug metabolizing enzymes. The altered metabolism of liver due to partial hepatectomy may also be responsible for the alterations in the renal enzymes.

The unaltered level of hepatic glutathione with 50 $\mu$ mole nickel is not parallel with the previous reports\textsuperscript{6) where a depletion in its level at 6 hrs with a rebound increase at 16 hrs have been observed after administering 250 $\mu$ mole nickel. The unaltered level of glutathione in our experiment could be due to a low dose of administered nickel. This is further supported by the induction in hepatic glutathione level at a higher dose in our experiment. The enhancement in hepatic glutathione level at a higher dose in both hepatectomized and unoperated rats may be due to nickel stimulation of synthesis of the tripeptide which in turn could be essential for maintaining sulfhydryl containing proteins involved in the detoxication process.\textsuperscript{6)}

Glutathione-S-transferase is an enzyme responsible for conjugation of glutathione with xenobiotics during the first step in the biosynthesis of mercapturic acid. The induced activity of glutathione-S-transferase along with the enhanced level of glutathione as a result of nickel administration suggests that it does not affect the biotransformation and elimination of various xenobiotics. However, the biological impact of chemicals which are detoxified or metabolically transformed by cytochrome P-450 system is greatly altered by nickel.\textsuperscript{8)} The concomitant increase in glutathione-S-transferase and glutathione could have adaptive significance. It is known that a number of compounds are biologically inactivated as a result of their conjugation with glutathione in presence of glutathione-S-transferase. Since nickel by increasing the rate of heme degradation diminishes the ability of cells to detoxify chemicals, the increase in glutathione and glutathione-S-transferase would thus partially compensate for nickel induced impairment of cytochrome P-450 dependent detoxication process. Since nickel also induces heme oxygenase an -SH dependent enzyme, the increased level of glutathione could protect the thiol moiety of protein and thus prevent its inactivation.\textsuperscript{8)}

The enhancement in the hepatic and renal phosphoglucomutase might lead to the fast turnover of NADPH in the system due to the increased turnover of the substrate, glucose-6-phosphate. NADPH is known to maintain the redox potential of the system including the level of reduced glutathione. Since nickel impairs the drug metabolizing enzymes including cytochrome P-450, an increased turnover of NADPH may partially compensate the detoxication of nickel in the biological system.

From the above discussion, it is concluded that in addition to reduced
glutathione and glutathione-S-transferase which are known to play a vital role in the detoxication of various xenobiotics including heavy metals, the enhanced level of phosphoglucomutase might play an additional role in the generation of NADPH, which will not only maintain the level of glutathione but also the redox potential of the biological system in general.

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