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·*Original Article*·

Alterations in hepatic metabolism of adult male rats following exposure to hydroxyprogesterone during embryonic development

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Abstract

Aim: To investigate the effect of *in utero* exposure to hydroxyprogesterone (HP) on liver metabolism in adult male albino rats. **Methods:** Pregnant Wistar strain albino rats were exposed to supra-normal levels (10 mg/kg and 25 mg/kg) of HP on days 1, 7 and 14 of pregnancy. The male pups were maintained under controlled conditions and the rats were killed 90 days after birth. The liver tissue was immediately excised, weighed and used for biochemical assays. **Results:** The activity levels of succinate dehydrogenase (SDH), glutamate dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G-6-PDH), malate dehydrogenase (MDH) and aminotransaminases were significantly increased in the livers of rats exposed to HP during embryonic development. The lactate dehydrogenase (LDH) activity level was significantly decreased in the liver of experimental rats. Furthermore, there was a significant elevation of activity levels of antioxidant enzymes (glutathione S-transferase [GST] and catalase [CAT]) with an increased lipid peroxidation in the hepatic tissue of experimental rats compared with the control group. **Conclusion:** The results of the present study suggest that there is an increase in the oxidative metabolism, antioxidative mechanism and levels of lipid peroxidation in rats exposed to HP during embryonic development. The increased aminotransaminase activities in these rats reveal tissue damage and disruption of mitochondrial integrity. *(Asian J Androl 2006 Jul; 8: 463–467)*

Keywords: hydroxyprogesterone; liver; oxidative enzymes; antioxidants; lipid peroxidation; embryonic development

1 Introduction

Hydroxyprogesterone (HP) is one of the most effective and widely prescribed drugs in Andhra Pradesh, India to prevent abnormal uterine bleeding and threatened miscarriage in women. There is a growing concern that exposure to xenobiotic compounds capable of modulating or disrupting the endocrine system may have harmful consequences for human male reproductive health. Based on a meta-analysis of 61 studies, it was suggested that human sperm quantity and quality have decreased during the last 50 years [1]. Data have also suggested an increased incidence of certain human male reproductive tract abnormalities, such as cryptorchidism and hypospadias [2, 3] during the same period. It has been hypothesized that these reproductive abnormalities may have a common origin from the embryonic development and/or neonatal life. One ironic example is that the children

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of women treated with diethylstilbestrol (a synthetic estrogen that was formerly used by physicians as an antiabortive agent) are now suffering from reproductive abnormalities [4].

In view of this, an elaborate program was initiated to evaluate the effect of prenatal exposure to synthetic steroidal drugs on reproductive abnormalities in rats. We reported earlier that prenatal exposure to HP significantly decreased steroidogenic enzyme activity levels in testis of male rats [5], and histopathological studies revealed severe effects on the spermatogenesis process in the testis [6]. Rats exposed transplacentally to 25 mg HP/kg have significantly low circulatory testosterone levels [7]. Here we reported the alterations in the hepatic metabolism in adult male rats exposed to HP during embryonic development.

2 Materials and methods

2.1 Chemicals

The 17 alpha-hydroxyprogesterone caproate (Proluton Depot®, German Remedies, Goa, India), commonly prescribed to prevent threatened miscarriage in women is available in an oily solution (250 mg in 1.0 mL) of castor oil Indian Pharmacopoiea (IP) and benzyl benzoate IP (1:1.7).

2.2 Maintenance of animals and treatment

Albino rats of the Wistar strain bred in the animal facility of the Department of Biotechnology, Sri Venkateswara University, Tirupati, were used. The rats were maintained under a regulated light : dark (12 h:12 h) schedule and were provided food and water *ad libitum*. The rat feed was purchased from Sai Durga feed agencies (Bangalore, India). Only adult rats (90 days old) were used in the present study. The experiments were conducted in accordance to the regulations of the University Ethical Committee and complied with the laws of the country.

The rats were allowed to mate and the pregnant rats were divided into three groups with 10 animals in each. The animals in group 1, which served as the control, were treated the same as those in the two experimental groups but received an injection of 20 μL mixture of castor oil and benzyl benzoate (1:1.7). The rats in groups 2 and 3 received intraperitoneal injection of 10 mg/kg or 25 mg/kg 17 alpha-HP caproate, respectively, on days 1, 7 and 14 of pregnancy. The male pups were maintained under controlled conditions and were weighed and killed 90 days after birth. The liver tissue was immediately excised, blotted on filter papers, weighed wet and used for biochemical analysis.

2.3 Methods

Liver tissue homogenates (10% w/w) were prepared as follows: in 0.25 mol/L ice-cold sucrose solution for all dehydrogenase enzyme assays; in 50 mmol/L phosphate buffer (pH 7.0) for catalase assays; in 50 mmol/L Tris-HCl (pH 7.4), containing 1 mmol/L ethylene diamine tetra acetic acid (EDTA) and 1 mmol/L diethyl dithiocarbamate for glutathione S-transferase (GST); and in 1.15% potassium chloride solution for lipid peroxidation. The mitochondrial and the cytosol fractions were separated by centrifugation and used for biochemical analysis. Succinate dehydrogenase (SDH) (EC: 1.3.99.1) and malate dehydrogenase (MDH) (EC: 1.1.1.37) were assayed using the method described by Nachlas *et al.*[8], lactate dehydrogenase (LDH) (EC: 1.1.1.27) by the method of Sreekanthan and Krishnamurthy [9], glucose-6-phosphate dehydrogenase (G-6-PDH) (EC: 1.1.1.49), aspartate aminotransaminase (AAT) (EC: 2.6.1.1) and alanine aminotransaminase (AlAT) (EC: 2.6.1.2) by the method of Bergmeyer and Bernt [10], isocitrate dehydrogenase (ICDH) (EC: 1.1.1.41) by the method of Kornberg and Pricer [11], glutamate dehydrogenase (GDH) (EC: 1.4.1.3) by the method of Lee and Lardy [12], glutathione S-transferase (GST) (EC: 2.5.11.8) by the method of Habig *et al*. [13], catalase (CAT) (EC: 1.11.1.6) by the method of Chance and Machly [14], and lipid peroxidation levels were determined by the method of Ohkawa *et al.* [15]. Protein content in the enzyme source was determined using Folin phenol reagent [16]. Enzyme activity was expressed in standard units, that is, μmol of product formed or substrate cleaved/mg protein/h. The rate of lipid peroxidation was expressed as μmol of malondialdehyde formed/g wet weight of tissue.

2.4 Statistical analysis

The data were presented as mean ± SD and analyzed using unpaired *t*-test [17]. Significance of difference was set at $P < 0.05$.

3 Results

The activity levels of SDH, ICDH, MDH, GDH and G-6-PDH were significantly increased (40.36%, 98.04%,

45.42% and 101.16%, respectively) in the liver tissue of rats exposed to HP *in utero* (Figure 1). In contrast, the LDH activity level was significantly decreased (42.60%) in the livers of HP-exposed rats when compared with control rats (Figure 1).

AAT and AlAT activity levels were significantly increased (25.48% and 21.88% respectively) in the liver of *in utero* HP-exposed rats compared with the control rats (Figure 2).

The activity levels of CAT and GST in the liver tissue of rats exposed to HP *in utero* was significantly higher when compared with the corresponding controls and the levels of lipid peroxidation products also increased significantly in experimental rats when compared with the control rats (Figure 3).

4 Discussion

It is evident that *in utero* HP exposure has a marked effect on the oxidative metabolism of the rat. In the present study, the Krebs cycle (represented by SDH, ICDH and MDH), the hexose monophosphate (HMP) pathway (represented by G-6-PDH) and glycolytic pathway (represented by LDH) all showed significant alterations in rats exposed to HP *in utero*. This is also the case with the enzymes connected with nitrogen metabolism, namely AAT, AlAT and GDH. The anti-oxidant enzyme activity levels (represented by GST and CAT) increased in the livers of rats exposed to HP during embryonic development. Similar results were observed in the livers of rats exposed to Phenobarbital [18].

The increased SDH activity implies increased channeling of pyruvate by way of the Krebs cycle. The results clearly indicate that the energy production through aerobic oxidation is increased in HP-exposed rats. Isocitrate dehydrogenase catalyses the reversible oxidation of isocitrate to oxalosuccinic acid, followed by decar-boxylation, leading to the formation of α-ketoglutarate. Nicotinamide adenine dinucleotide-ICDH is found only in the mitochondria and this enzyme appears to participate in the tri carboxylic acid (TCA) cycle. MDH is a principal member of the TCA cycle enzymes, which uses NAD as a cofactor and catalyses malate to oxaloacetate. Increased activity levels of SDH, ICDH and MDH indicate increased energy output in the experimental rats.

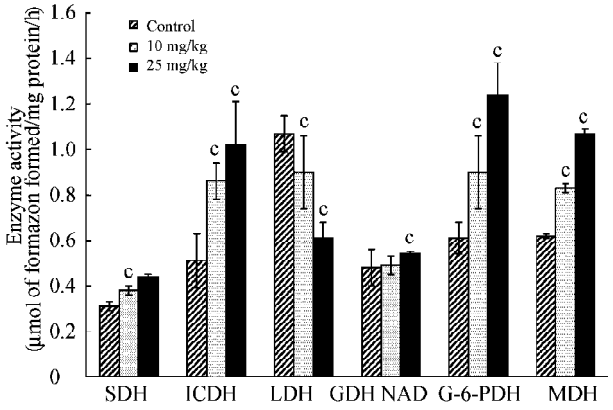

Figure 1. Changes in the activity levels of selected dehydrogenase enzymes, succinate dehydrogenase (SDH), NAD-isocitrate dehydrogenase (ICDH), lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G-6-PDH) and malate dehydrogenase (MDH), in livers of male rats exposed to HP during embryonic development. Mean ± SD (n = 10). $^cP < 0.01$, compared with the control. NAD, Nicotinamide adinine dinucleotide.

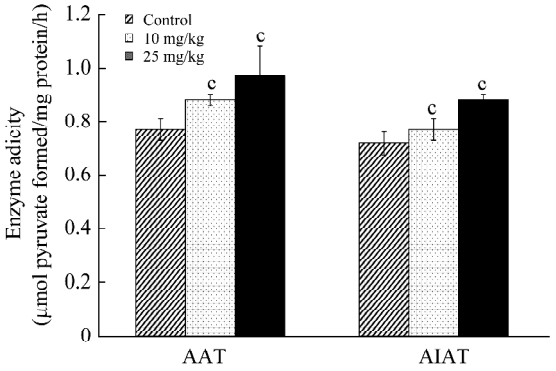

Figure 2. Changes in the activity levels of aspartate aminotransaminase (AAT) and alanine aminotransaminase (AlAT) in livers of male rats exposed to HP during embryonic development. Mean ± SD (n = 10). $^cP < 0.001$, compared with the control.

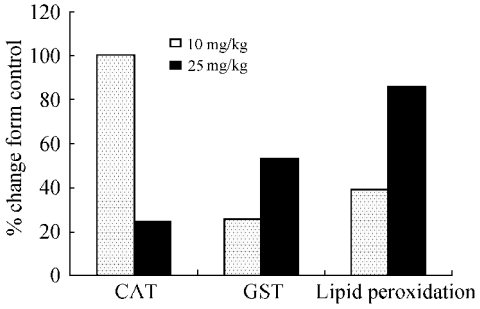

Figure 3. Changes in the activity levels of catalase (CAT), glutathione S-transferase (GST) and lipid peroxidation in the livers of male rats exposed to HP during embryonic development. The data represents % change in enzyme activity from controls (n = 10).

G-6-PDH is a representative of the HMP shunt pathway and operates at the critical diversion point of the pentose phosphate pathway from glycolysis. An increase in G-6-PDH activity was observed in the liver tissue of albino rats as a consequence of HP exposure. This alteration may indicate an increased operation of the HMP pathway, and the increased G-6-PDH activity facilitates the increased production of nicotinamide adinine dinucleotide phosphate reduced form (NADPH) for the detoxification process.

LDH plays an important role in carbohydrate metabolism and catalyses the inter-conversion of lactate and pyruvate [19]. In the present study, the lowered LDH activity levels after administration of HP clearly indicates decreased conversion of pyruvate to lactate. Transaminases (AAT and AlAT) are intracellular enzymes which operate at the critical crossroads of carbohydrate metabolism and protein metabolism by inter-converting α-ketoglutarate, pyruvate and oxaloacetate on one side, and alanine, aspartate and glutamate on the other. Tissue damage or disruption of mitochondrial integrity may cause an increase in AAT and AlAT activity levels. Increased transaminase activity has also been reported during xenobiotic stress [20, 21].

In the present study, the alterations in CAT and GST activity levels and the levels of lipid peroxidation product in the livers of *in utero* HP-treated rats indicated the stress of the chemical on antioxidation. CAT acts on hydrogen peroxides generated either through the metabolism of endogenous substances or the metabolism of exogenous compounds. Because it removes reactive hydrogen peroxide from the cell, it is important in the detoxification mechanism. CAT is very active in removing peroxy radicals and is able to protect cells from injury. Increased activities of CAT and GST in experimental rat tissue may serve as a physiological adaptation during experimental conditions. The glutathione-dependent enzymes are involved in scavenging the free radicals in the tissues, thereby blocking the propagation of lipid peroxidation. The increased activity levels of GST and CAT eliminate the highly reactive free radicals and serve as a defense mechanism [22].

Malondialdehyde, a lipid peroxidation product generated in tissues by free radical injury, is measured by thiobarbituric acid reactivity and is considered a sensitive index of free radical generation [23]. In the present study, the elevated lipid peroxidation was used as an index of oxidative stress caused by HP. Some studies have reported increased lipid peroxidation in epididymal tissue under xenoestrogen stress [24–26].

It can therefore be concluded that the experimental rats in our study appear to be meeting their energy demands through the operation of the HMP pathway, as reflected by elevated G-6-PDH activity, and through the TCA cycle, as indicated by SDH, ICDH and MDH activity levels. The findings reported here also suggest that HP-stimulated lipid peroxidation and increased GST and CAT enzyme activities may be viewed as a protective mechanism to counteract the peroxide tone. Thus the results of the present study indicate that HP exposure during embryonic development not only causes reproductive abnormalities in adult male rats [5–7], but also alters hepatic metabolism.

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