

Estradiol Effect on the Glucose-6-Phosphate Dehydrogenase Activity: an *in vivo* Study

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Abstract

In the present study, the activity of glucose-6-phosphate dehydrogenase in the liver of rats treated intraperitoneally (ip) with three different doses (50, 100, 200 µg/Kg rat) of estradiol-17β (E₂), over a period of 10 days has been assessed. A significant increase in glucose-6-phosphate dehydrogenase activity was recorded in all treated groups compared to controls. The results reveal that such an increase is positively proportional to the used dose:

- * With the dose of 50 µg E₂/kg rat, the increase was recorded from the fourth injection.
- * With the dose of 100 µg E₂/kg rat, the increase was recorded from the second injection.
- * With the dose of 200 µg E₂/kg rat, the increase was recorded from the first injection.

Our results suggest that the increase in glucose-6-phosphate dehydrogenase activity in treated rats increases its capacity in generating NADPH, the co-enzyme indispensable for the reduction of estradiol-17β metabolites.

Key words : Glucose-6-phosphate dehydrogenase, Detoxification, Estradiol-17β, Glutathione, Rats.

Résumé

Nous avons étudié le glucose-6-phosphate dehydrogenase dans le foie des rats traités avec trois doses différentes (50, 100 et 200 µg/kg rat) de 17 β-oestradiol (E₂) à travers l'administration d'injections intra péritonéales (ip) données tous les deux jours, sur une période de 10 jours. Une augmentation significative de l'activité de la glucose-6-phosphate dehydrogenase a été signalée au niveau de tous les groupes des rats traités, comparativement avec les témoins. Les résultats révèlent que chaque augmentation est positivement proportionnelle avec la dose utilisée:

- * Avec la dose de 50 µg E₂/kg rat, l'augmentation a été remarquée à partir de la quatrième injection.
- * Avec la dose de 100 µg E₂/kg rat, l'augmentation a été remarquée à partir de la deuxième injection.
- * Avec la dose de 200 µg E₂/kg rat, l'augmentation a été remarquée à partir de la première injection.

Nos résultats suggèrent que l'activité de la glucose-6-phosphate déshydrogenase chez les rats traités, confirme sa capacité à générer le NADPH, le co-enzyme indispensable à la réduction des métabolites de la 17β-oestradiol.

Mots clés: Glucose-6-phosphate dehydrogenase, Détoxication, Oestradiol-17β, Glutathion, Rats.

INTRODUCTION

Natural or synthetic estrogens are known to cause biochemical changes at cell levels, tissues, and organs in laboratory animals [1]. *in vivo* changes in estradiol-17 β (E₂) provoke carcinogenic metabolites of the catecholestrogens, such as 2-hydroxyestradiol and 4-hydroxyestradiol. Following oxidation, these toxic metabolites give rise to semiquinones and quinones, throughout oestrogen redox cycle reactions [2]. The target organ of such toxic metabolites is the liver, with values 2.5 times higher than those of kidney. Such metabolites generate a number of free active radicals, that are highly damaging for proteins and DNA [3], leading to carcinogenic tumours of liver and kidney cells, testicles, uterus and breast [4]. They also induce some pathologies gallbladder pathologies, particularly gallstone [5].

The glucose-6-phosphate dehydrogenase (G6PD) [D-glucose-6-phosphate/NADP⁺ oxidoreductase, EC: 1.1.1.49], which is present in all living cells, is of a capital importance as it is the key enzyme of the pentose phosphate pathway [6]. It also plays a major role in protecting animal cells from oxidizing factors, via production of the co-enzyme NADPH+H⁻: (reduced from of Nicotinamide Adenine Dinucleotide Phosphate) [7], which takes part in most of the oxido-reduction reaction within the living organism, through glutathione redox cycle [8].

The glutathione peroxidase (GPx) [EC: 1.1.10.9] is the first enzyme in the glutathione redox cycle involved in the reduction of both organic and inorganic peroxides, using reduced glutathione (GSH). The second enzyme in glutathione redox cycle is the glutathione reductase (GR) [EC1.6.4.2]; that restores the reduced cellular milieu, and maintains the normal GSH/GSSG ratio, thus catalysing redox cycle reactions of oxidized glutathione (GSSG) to reduced glutathione (GSH), in the presence of the co-enzyme NADPH+H⁺ that is produced by the glucose-6-phosphate dehydrogenase (Fig. 1).

Due to the vital anti-oxidative role of the G6PD in restoring and maintaining the reduced cellular medium, and hence, protecting cells from oxidative reactions, the present investigation has been carried out to illustrate the capacity of such an enzyme in the detoxification of estradiol metabolites in rats.

MATERIALS AND METHODS

All chemicals used to perform this work have been purchased from *Sigma Chemical Company*. 120 albino male wistar rats of 11 weeks of age and an average weight of 250 g were brought from *Pasteur Institute (Algeria)*. Animals were housed in 20 cages. They were located in a room with an ambient temperature of 21 $\pm$ 1 C° and to a photoperiod of 12 hours of light per day. They were fed on a standard

specified diet with free access to water. They were then divided into 4 groups of 30 each. The control group was injected intraperitoneally (ip) with 0.1 ml/rat of olive oil (OO) only, while the three other groups received ip injections of 50, 100 and 200 μ g E₂/kg rat, respectively. Estradiol-17 β was firstly dissolved in 0.1 ml of olive oil. The experiment lasted for 10 days with injections made at 2 days interval. However, six individuals from each group have been killed by decapitation every 2 days, following the start of the treatment. Immediately after each decapitation; the livers were excised for G6PD activity measurement according to the procedure of Löhr and Waller [9]. Protein concentrations were also measured according to Bradford method [10]. Data were statistically analysed using the Fisher test (with two variances), followed by Student *t*-test.

RESULTS

Table 1 shows changes in glucose-6-phosphate dehydrogenase activity in the rat liver in all groups. Following the first injection (2 days), the glucose-6-phosphate dehydrogenase activity increased significantly ($P<0.01$) in rats receiving 200 μ g E₂/kg, with a percentage of 42.8 %, compared to controls. Yet, after the second injection (4 days), such a significant increase ($P<0.01$) was observed in rats treated with 100 and 200 μ g E₂/kg, with an increase percentages of 27 and 41.6 %, respectively, compared to controls. Similarly, a rise in G6PD activity was recorded only in the two previous groups after the third injection (6 days), with percentages of 28 and 50.3 % respectively, always in comparison with controls. However, following the fourth injection (8 days), all treated rats showed a remarkable increase in G6PD activity ($P<0.01$), with percentages varying from 27.7 % for those receiving 50 μ g E₂/kg, 44 % for those receiving 100 μ g E₂/kg and 63 % for individuals receiving 200 μ g E₂/kg, compared to control group. Finally, at the end of the experiment (10 days), the percentage of G6PD activity was much more pronounced in all treated groups with the highest ratio of 71 % ($P<0.001$) recorded in rats treated with 200 μ g E₂/kg.

DISCUSSION

The present results demonstrate that the glucose-6-phosphate dehydrogenase activity in the liver of the controls (injected with olive oil) did not change along the experimental period. This asserts that the olive oil did not produce any toxic metabolites. Moreover, they also show that there is a dose-dependent effect throughout the 10 days experimental period. Such observations confirm those made by Smith and Barker [11] on the effect of estradiol-17 β on glucose-6-phosphate dehydrogenase

activity in rats. Besides, it seems clear that *in vivo* treatment with estradiol leads to the formation of a number of toxic compounds via estrogen redox cycle [2], which, in turn, enter the detoxifying reactions through glutathione redox cycle [8]. It is worth mentioning that, through the mediation of the glutathione reductase and its co-enzyme NADPH (one of the important products of G6PD), most of the reduced glutathione regenerates as a result of reducing reactions. These observations reinforce the possible role of the G6PD in the protection of living cells from oxidative damages [7]. Similar conclusions have been made on patients suffering from deficiency in glucose-6-phosphate dehydrogenase, due to genetic factors, following fava beans consumption (rich in divicine) require more cell glutathione to prevent haemolytic anaemia [12]. It is, thus, clear that the regeneration of reduced glutathione requires a very important G6PD activity [7]. Our results convey those of Ham & Liehr [3], in which an increase ratio of 34 % in the G6PD activity in rat liver treated (*in vitro*) with T-butylhydroperoxide has been reported. In conclusion our results asserts that the liver is an estradiol target organ, and the G6PD plays a very important role in protecting cells and tissues from oxidative agent, via the production of NADPH, which is required for the oxido-reduction reactions of most toxic metabolites.

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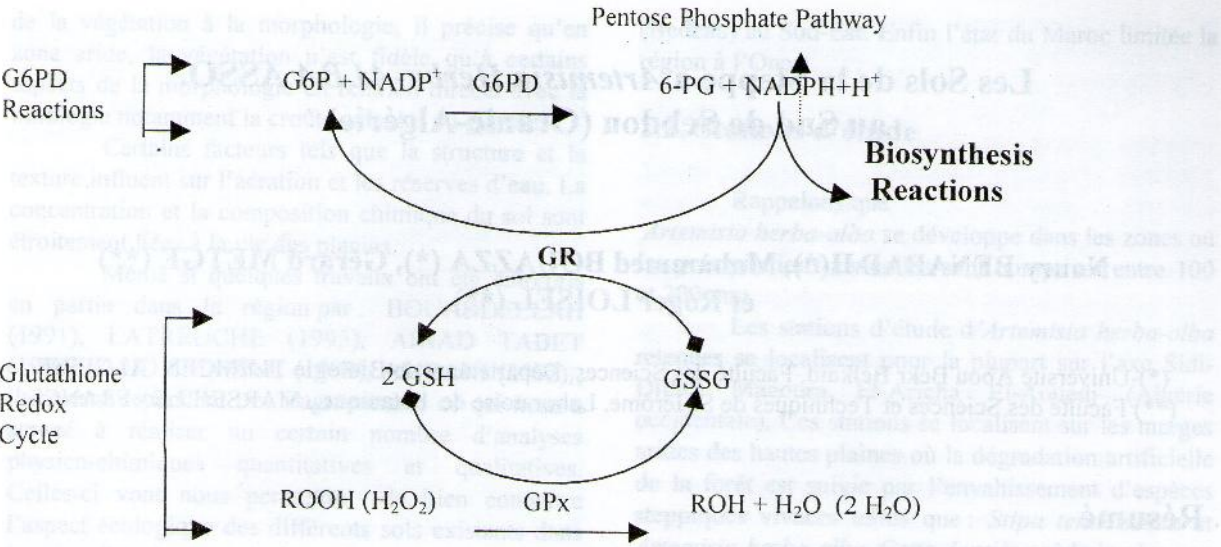


Figure 1: Physiological role of G6PD reaction, and its relationship with detoxifying process via glutathione redox cycle. The two products of G6PD reaction are 6-PG and NADPH+H⁺. The first is a precursor of pentose phosphate pathway, whereas the second regenerates GSH, which enters the detoxifying reactions of most of toxic compounds via glutathione redox cycle. (NADPH+H⁺ is needed for variety of biosynthesis reactions)

Table 1:
Glucose-6-phosphate dehydrogenase (G6PD) activity (U/mg protein) in rat liver after 2, 4, 6, 8 and 10 days of treatment with 0.1 ml olive oil (control) and with three doses of estradiol-17B (50, 100 and 200µg/kg rat).

Days of injection	D. Control (olive oil)	E. Treatment with estradiol-17B		
		50 µg/kg	100 µg/kg	200 µg/kg
2	198.1 ± 8.6	216.6 ± 8.7 (9.3)	236.8 ± 12.4 (19.5)	283.0 ± 18.0 ** (42.8)
4	201.9 ± 12.8	226.4 ± 7.4 (12.1)	256.4 ± 15.9 * (27.0)	285.9 ± 11.8 ** (41.6)
6	200.1 ± 12.8	240.9 ± 15.0 (20.3)	256.5 ± 13.2 * (28.1)	300.8 ± 23.8** (50.3)
8	202.8 ± 10.9	259.1 ± 10.1 * (27.7)	291.9 ± 21.1 ** (43.9)	331.0 ± 22.2 ** (63.2)
10	203.8 ± 11.6	277.7 ± 24.5 * (36.2)	295.7 ± 19.1 ** (45.0)	348.5 ± 15.6 *** (71)

Values are given as mean ± SEM (n = 10). Values indicated between parentheses represent percentage of G6PD activity compared to their corresponding controls.
* (P < 0.05) from control group.
** (P < 0.01) from control group.
*** (P < 0.001) from control group.