

Immunolocalization of Glutathione-Peroxidase (GPx1) in the Rat Adrenal Cortex: Correlation between Steroidogenesis and Lipid Peroxidation

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In order to confirm the relationship between glutathione-oxidase (GPx1) and biological significance on steroidogenesis, we have studied the immunocytochemical localization of GPx1 in the rat adrenal cortical cells. GPx1 was observed not only in cytoplasm (cytosol GPx1) but in mitochondria (mitochondrial GPx1). The staining intensity was altered by the functional state of the adrenal cortical cells. Furthermore, cytosol- and mitochondrial-GPx1 was modified by lipoperoxidative damage in the adrenal cortical cells. Therefore, we proposed that the pattern of GPx1 staining should be a more sensitive and specific indicator of oxidative damage in cells. Thus, the staining pattern of GPx1 is thought to be a useful marker for lipid peroxidation in the adrenal cortical cells.

Key words: glutathione-oxidase (GPx1), adrenal cortical cell, mitochondria, steroidogenesis, rat

I. Introduction

Glutathione-oxidase (GPx1) which effectively reduces the lipid peroxides is a selenium-dependent enzyme that exists as a homotetramer with each 22-kDa subunit containing a selenium atom incorporated within a catalytically active selenocystein residue [6]. There are three other members of the selenium-dependent GPx1 family, although cytosolic GPx1 is the predominant form [8, 51].

The gene encoding GPx1 is mapped on chromosome 3q 11-13 [5]. Because GPx1 decomposes hydrogen peroxide and organic hydroperoxides produced during normal metabolism and after oxidative insults, GPx1 prevents peroxide-induced DNA damage, lipid peroxidation, and protein-degradation [13, 55]. P53, after being activated by DNA-damaging reagents, has been shown either to induce G1 growth arrest or apoptosis [21]. The P53 target genes that mediate or associate with p53-induced apoptosis in-

duces Bax [20], Fas/APO 1 [13], as well as those involving generation of reactive oxygen species [43]. Tan *et al.* [49] have identified and characterized GPx1, an antioxidant enzyme, as also induced by p53. It appears paradoxical that p53, on the one hand, induces the gene responsible for reactive oxygen species generation, which mediates apoptosis [43], and on the other hand, induces expression of the protective antioxidant enzyme GPx1, which protects cells from oxidative damage and apoptosis [23]. It is known that p53-induced reactive oxygen species generation is a rather latter event [43]. Thus, p53 may regulate cellular redox status in a time-dependent manner; it increases antioxidant synthesis at an early stage followed by an increase in reactive oxygen species generation.

The significant increase of GPx1 protein triggered by lipid peroxides has been demonstrated in an experimental system of inactivation and reactivation of the arachidonate cascade in rat peritoneal macrophages [35, 55]. These findings indicate that increased levels of lipid peroxides (or peroxidation) enhance the expression of GPx1, or, in other words, a decrease in lipid peroxides likely reduces the expression of the enzyme. Lowed lipid peroxidation in cancer cells has been detailed by several investigators [2,

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Table 1. Immunolocalization of glutathione-peroxidase (GPx1) in the rat adrenal cortical cells and lipid peroxides

	Light microscopy	Electron microscopy	Lipid peroxides
control	three zones	cytosol (lipid-laden clear cells) mitochondria (lipid-depleted compact cells)	lipid hydroperoxides steroid hydroperoxides
HX	atrophic zones (inner layer of zonae fasciculata and reticularis)	cytosol (around the accumulated lipid droplets)	lipid hydroperoxides steroid hydroperoxides
HX + ACTH	three zones	mitochondria (lipid-depleted compact cells)	steroid hydroperoxides
Elipten	zona fasciculata	cytosol (around the accumulated lipid droplets) mitochondria (a few number)	lipid hydroperoxides steroid hydroperoxides
4-APP	inner layer of zonae fasciculata and reticularis	cytosol (near the smooth endoplasmic reticulum)	phospholipid hydroperoxides
Metopiron	outer fasciculata	cytosol (near the mitochondria or lipid droplets) mitochondria (a few number)	lipid hydroperoxides steroid hydroperoxides

HX: Hypophysectomy, Elipten: Aminoglutethimide, 4-APP: 4-aminopyrazolopyrimidine, Metopiron: 2-methyl-1-1,2-bis(3-pyridyl)-1-propanone.

52] and has been related to changes in fatty acid composition [7]; anti-oxidant such as vitamin E [7] and a reduced form of glutathione [12]; and enzyme activities of lipid peroxide-scavenging enzymes, including GPx1 [9, 18] and glutathione-S-transferase [27, 41]. Therefore, the suppressed expression of GPx1 in cancer may be related to the low amount of lipid peroxides within the cell. A large number of studies have been reported on the role of fulfilled by GPx1 in the protection of various tissues against lipid peroxidation [29–33, 35–40, 55–57]. Furthermore, GPx1 staining was modified by lipoperoxidative damage in tissues or cells. Therefore, we propose that the pattern of GPx1 staining should be a more sensitive and specific indicator of oxidative damage in tissues [29–33, 35–40, 55]. The staining pattern of GPx1 is thought to be useful marker for lipid peroxidation in the cells.

In this review we focus on the intracellular localization of GPx1 in the rat adrenal cortical cells.

II. Intracellular Localization of GPx1 in the Rat Adrenal Cortical Cells

Normal untreated

Immunohistochemical localization of GPx1 is observed in the cortical cells in all three zones of the adrenal cortex. No GPx1 is seen in the medulla. In immunoelectron microscopic investigations, GPx1 is observed not only in cytoplasm (cytosol GPx1) but also in mitochondria (mitochondrial GPx1) in the rat adrenal cortical cells (Table 1). Cytosol GPx1 is mainly observed in lipid-laden outer fasciculata cells. Mitochondrial GPx1 which is present in the intramitochondrial cristae, is mostly observed in lipid-depleted compact cells inner part of zona fasciculata [31]. Biochemically, steroid hydroperoxides has been detected in cytosol and mitochondrial fractions [29].

Hypophysectomy

In the hypophysectomized rat adrenal gland, atrophy is most conspicuous in the inner layer of zonae fasciculata and reticularis. GPx1 is mainly localized in the cells of those atrophic cortical zone cells (Table 1) [29, 31]. Under

this condition, cytosol GPx1 around the accumulated lipid droplets are predominant and no mitochondrial GPx1 is detected (Table 1). Those cells in the atrophic zone also exhibit cytoplasmic accumulation of lipids. Increased steroid hydroperoxides in the adrenal glands of hypophysectomized rats are also demonstrated [29]. From these findings, it is quite conceivable that the accumulated lipids in the cells of zonae fasciculata and reticularis, ACTH-dependent zones, of hypophysectomized rats would be peroxidated by either microsomal or mitochondrial oxidation system, and GPx1 could be working on reduction of the lipid peroxides in those cells. Actually, it was proved that the liver GPx1 was playing an important role in preventive and repair process of lipoperoxidative hepatic injuries [55–57].

ACTH-administration

By ACTH-administration to the hypophysectomized rats, GPx1 is observed in the cortical cells in all three zones of the adrenal cortex. The intensity of the immunohistochemical staining of GPx1 is stronger than in the case of the untreated rats [29, 31]. Under this condition, an increased number of mitochondrial GPx1 is clearly detected (Table 1). It is a well documented fact that zona fasciculata cells synthesize corticosterone as a final product in the rat adrenal cortex and cytochrome P-450 (P-450)_{11β} activity is mainly restricted to the zona fasciculata cells [28]. Mitochondrial GPx1 has been mainly observed in the adrenal cortical cells of the zona fasciculata [31]. Upon NADPH-dependent lipid peroxidation reaction, adrenal cortex mitochondrial P-450 has been destroyed in a parallel manner as the formation of a malondialdehyde [19]. The cytochrome responsible for 11β-hydroxylation has been more susceptible to degradation than for cholesterol side-chain cleavage reaction [19]. Timcenko-Youssef *et al.* [53] demonstrated that the adrenal cortical mitochondrial GPx1 protects the degradation of P-450 by lipid peroxidation. Therefore, it is strongly suggested that mitochondrial GPx1 may be a very important enzyme for the steroidogenesis, especially, corticosterone synthesis. In addition, mitochondrial GPx1 in the adrenal cortical cells is considered to be ACTH-dependent [29, 31–33, 38].

Aminoglutethimide (Elipten)-administration

Elipten is well known to inhibit the conversion of cholesterol into pregnenolone including cholesterol side chain cleavage system [3, 10, 25]. And this conversion occurs in mitochondria. Ultrastructurally, it has been reported that mitochondrial swelling and lipids stored in the adrenal cortical cells under the Elipten-administration [26, 44]. Immunohistochemically, GPx1 is mainly localized in zona fasciculata cells of the adrenal cortex (Table 1). Ultrastructurally, cytosol GPx1 around the electron dense lipid droplets is mainly observed [32]. And the intensity of the cytosol GPx1 staining is stronger than that of the untreated rats. These findings may suggest that accumulated lipids are peroxidized lipids. It is demonstrated that steroid hydroperoxides in the adrenal gland of the hypophysectomized rats are detected in cytosol fraction and that adrenal cortical cells contained many lipid droplets [29]. Therefore, GPx1 could be working on reduction of those accumulated lipid hydroperoxides [51]. On the other hand, immunocytochemically, GPx1 is also observed in the mitochondria [32]. However, the number of mitochondrial GPx1 is less than that in the untreated rats. Ultrastructurally, mitochondrial swelling or cavitation has been reported under the Elipten-treated condition [26, 44]. It may be in close relation to the mitochondrial peroxidation. In this viewpoint, mitochondrial GPx1 under the Elipten-administration may suggest an important role in prevention of damage to mitochondria with lipid peroxidation. In fact, lipid peroxidation correlated well with swelling, and finally with lysis and disintegration of the mitochondria [50].

4-aminopyrazolopyrimidine (4-APP)-administration

4-APP has been reported to inhibit hepatic release of lipoprotein and to decrease serum cholesterol levels remarkably [47]. And it has been known that lowered serum cholesterol levels in extrahepatic production of cholesterol including adrenal cortex [1]. Biochemically, the adrenal cortical cholesterol synthesis is known to increase 42 times more in the 4-APP-treated rats than the untreated rats [1]. Immunohistochemically, GPx1 is mainly observed in inner layer of zona fasciculata and reticularis (Table 1) [32]. Ultrastructurally, 4-APP-treated rats have almost complete depletion of cholesterol esters and proliferation of the smooth endoplasmic reticulum [34]. In these cells, immunocytochemically, GPx1 is mostly observed in cytosol near the smooth endoplasmic reticulum or mitochondria (Table 1) [32]. Immunocytochemical localization of GPx1 in the rat testicular Leydig cells is discussed and it strongly suggests that the very close relationship lies between the testicular GPx1 and membrane metabolism including reduction of lipid peroxides of well developed smooth endoplasmic reticulum [30]. Under the 4-APP-administration, cytosol GPx1 near the smooth endoplasmic reticulum is mostly observed. On acknowledging these observations, lipid peroxidation may occur in microsomes including smooth endoplasmic reticulum. It is well recognized that microsomal

membrane contain relatively large amounts of polyunsaturated fatty acid in their phospholipid and microsomes are very liable to lipid peroxidation and concurrent damage [45, 54]. Therefore, these phospholipids as membrane component are considered a major site of lipid peroxidation under the 4-APP-administration. GPx1 is thought to be involved in the reduction of the phospholipid hydroperoxides during membrane metabolism. In addition, no mitochondrial GPx1 is seen in the 4-APP-administration. It is suggested that mitochondrial GPx1 is dependent upon ACTH stimulation, *i.e.*, active steroidogenesis [29, 31, 38]. In this regard, adrenocortical mitochondria under the 4-APP-administration is thought to be rather inactive in steroidogenesis.

2-methyl-1-1,2-bis(3-pyridyl)-1-propanone (Metopiron)-administration

It is well recognized that Metopiron is an inhibitor for adrenocortical mitochondrial 11 β -hydroxylation (cytochrome P-450 11 β), which catalyzes the conversion of 11-deoxycorticosterone to corticosterone [4, 11, 46]. On the other hand, it is well known that Metopiron is the alteration in the outer membrane of the mitochondria and their degradation [17, 24, 48]. Immunohistochemically, GPx1 is mainly localized in the outer fasciculata and cytosol GPx1 near the mitochondria or lipid droplets is mostly observed (Table 1). However, mitochondrial GPx1 is less than that in the control rat adrenal cortical cell [33]. Mitochondrial GPx1 has been mainly localized in lipid-depleted compact cells of inner part of zona fasciculata [31]. It is generally accepted that zona fasciculata synthesize corticosterone as a final product in the rat adrenal cortex and cytochrome P-450 11 β activity is mainly restricted to the zona fasciculata [14–16, 28]. It is strongly suggested that mitochondrial GPx1 may be a very important enzyme for steroidogenesis, especially, corticosterone synthesis including cytochrome P-450 11 β . Thus, lipid peroxidation and subsequent lipid peroxides in the adrenal cortical mitochondria might occur following mitochondrial 11 β hydroxylation including cytochrome P-450 11 β . On the other hand, it is well known that Metopiron leads to an increased synthesis of deoxycorticosterone and 11-deoxycortisol [22]. Magalhaes *et al.*, reported that the decrease in the volume fraction of mitochondria and an increase in the volume fraction of the endoplasmic reticulum and the Golgi complex [25]. Thus, proliferated microsomes are thought to be hyperactive. As a result, cytosol GPx1 may be enhanced. This finding may correspond to the functional significance of the adrenocortical cells under Metopiron-administration.

III. Concluding Remarks

Immunocytochemical localization of GPx1 in the rat adrenal cortical cells is observed not only in cytoplasm (cytosol GPx1) but in mitochondria (mitochondrial GPx1). Cytosol GPx1 is found mainly in lipid-laden clear cells and

is intensity stained under low steroidogenic conditions. Mitochondrial GPx1 is detected mainly in lipid-depleted compact cells and depends on ACTH-stimulation; *i.e.*, active steroidogenesis. The possible role of adrenal cortical GPx1 is proposed as follows, A: reduction of lipid hydroperoxides or phospholipid hydroperoxides induced by cell injury (cytosol GPx1), B: reduction of steroid hydroperoxides produced during steroidogenesis (mitochondrial GPx1).

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