Immunolocalization of Glutathione S-transferase in the Rat Uterus

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Immunohistochemical localization of glutathione S-transferase (GST) and enzyme cytochemical staining for endogenous peroxidase (Px) activity were studied in rat uterus. Both enzymes were clearly detected in the endometrium of the uterus taken from proestrus to estrus during the estrous cycle. Based on our data, the biological significance of GST in endometrium was discussed.

(Key Words: Glutathione S-transferase, Endogenous peroxidase, Immunohistochemistry, Estrogen, Endometrium)

INTRODUCTION

Enzyme histochemical staining for endogenous peroxidase (Px) activity in the uterus is a specific marker for endometrium. Recently, Metzler et al (16) reported that endogenous Px of the uterus specifically binds DNA to estrogen in the presence of hydrogen peroxide. Hydrogen peroxide seems to be the agent responsible for initiating death in the cells, either directly or by initiation of lipid radical formation (18, 19, 21). On the other hand, depletion of cellular reduced glutathione, an important defense system against oxidants, caused more rapid lysis of the cells (2, 8, 22).

In the present study, we examined the immunocytochemical localization of glutathione S-transferase (GST) in the rat uterus and the change of its localization during the menstrual cycle. GST help detoxication by catalyzing the conjugation of many hydrophobic and electrophilic substances with reduced glutathione (14).

MATERIALS AND METHODS

1. Animals and tissue preparation

Adult female Sprague-Dawley rats (Charles River Japan Inc. Atsugi) were housed in air-conditioned quarters (22 ± 2°C) with rat chow and tap water ad libitum. Only rats that had shown two consecutive 4-day estrous cycle, as judged by vaginal smears, were used in this study. Each rat was killed at 2 to 6 hr intervals from 0300 hr of metestrus through midnight of estrus. Then, small pieces of uteri were fixed in periodate-lysine-4% paraformaldehyde solution (4%PLP) (15) for 18 to 20 hr at 4°C under constant agitation. The fixed tissues were then washed in 0.01M phosphate buffered saline (PBS), pH 7.4, containing from 10% up to a final 20% sucrose concentration overnight at 4°C. Subsequently, 6μm frozen sections were prepared from these tissues in a cryostat, and were placed on albumin-coated glass slides. The sections were washed in 0.01M PBS, pH 7.4, and immunohistochemical staining was performed by the indirect peroxidase-labelled antibody method after Nakane (17).

2. Immunohistochemical staining

The sections were pre-treated with 0.005M periodic acid for 15 min and 0.05M sodium borohydride for 30 min to inactivate endogenous peroxidase (4). Rabbit antiserum

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against rat glutathione S-transferase YP (GST; YF/subunit 7) was purchased from Bio Prep (Stillorgan Industrial Park, Stillorgan Co. Dublin, Ireland). This serum at 1:100 dilution was incubated on the sections at room temperature for 3 hr. As an immunologically negative control, 1:100 diluted nonimmune rabbit serum was used. Then, the sections were incubated with horseradish peroxidase-labelled anti-rabbit IgG Fab fragment of goat IgG for 3 hr at room temperature. After the incubation was completed, the sections were incubated in Graham-Karnovsky's reaction medium (6) which contained 20 mg% 3,3'-diaminobenzidine-tetrahydrochloride (DAB; Wako Pure Chemical Industries, Osaka) and 0.005% hydrogen peroxide in 0.05M Tris-HCl buffer (pH 7.6) for 5 to 10 min at room temperature. Then the sections were counterstained for nuclei with 1% methyl green dissolved in veronal acetate buffer (pH 4.2).

3. Enzyme cytochemical staining for endogenous peroxidase (Px) activity

4% PLP fixed and 6 μm frozen sections were used. The sections were incubated in Graham-Karnovsky's reaction medium (6) which contained DAB and 0.005% hydrogen peroxide as the substrate, for 10 min. Then the sections were counterstained for nuclei with 1% methyl green dissolved in veronal acetate buffer, pH 4.2. For electron microscopical observation of endogenous Px activity, the sections were pre-incubated for 30 min in Graham-Karnovsky's reaction medium (6) from which the substrate hydrogen peroxide was omitted and then they were incubated in the fully equipped reaction medium for 30 min. The sections were post-fixed in 1% O₃O₄ in 0.1M sodium phosphate buffer, pH 7.4, for 1 hr, dehydrated in graded ethanol series and embedded in Quetol 812 by an inverted gelatin capsule method. Ultrathin sections were prepared with LKB ultramicrotome and were observed under a JEOL 1200 EX electron microscope.

RESULTS

In the uteri taken from proestrus to estrus, immunohistochemical localization of GST was predominantly observed in the endometrium but not in the uterine gland or in interstitial tissues. At higher magnification, GST was diffusely distributed in the cytoplasm of these endometrial epithelial cells (Fig. 1). The con-

Fig. 1 Immunohistochemical localization of GST in the rat uterus taken from proestrus to estrus. GST is predominantly observed in endometrium. Peroxidase-labelled antibody method, × 150

Fig. 2 Using control serum (normal rabbit serum), no immunohistochemical reaction is noted in the rat uterus. Peroxidase-labelled antibody method, × 150

Fig. 3 Enzyme histochemical staining for endogenous Px activity in the rat uterus taken from proestrus to estrus. Endogenous Px is clearly detected in endometrium. (arrows: granulocytes) × 150
Fig. 4  Enzyme cytochemical localization of endogenous Px. Endogenous Px is noted in cisternae of rough endoplasmic reticulum and perinuclear space.

Bar = 1μm, ×10,000
control serum (NRS) was negative for immuno-
histochemical localization of GST in the rat
uterus (Fig. 2). At the same time, enzyme
histochemical staining for endogenous Px ac-
tivity was also detected in endometrial epithelial
cells (Fig. 3). By electron microscopical obser-
vations, endogenous Px was found in cisternae
of rough endoplasmic reticulum and in the
perinuclear space (Fig. 4). The nucleus showed
remarkable identation. The Golgi complexes
usually lacked endogenous Px.

DISCUSSION

In the present study, we found that immuno-
histochemical localization of GST in the rat
uterus was observed in the endometrium. Fur-
thermore, GST-positive endometrium was de-
tected in the uterus taken from proestrus to
estrus during the estrous cycle. Therefore, it is
thought to be an endometrium in a prolifera-
tive and/or secretory phase.

On the other hand, enzyme histochemical
staining for endogenous Px activity was also de-
tected in endometrium of the uterus taken from
proestrus to estrus during the estrous cycle. It
is a well documented fact that endogenous Px
activity of the uterus is induced by estrogen (1,
3). In addition, enzyme histochemical staining
for endogenous Px activity in endometrium of the
human uterus has been observed at proliferative
to secretory phase during the menstrual
cycle (9).

Based on our data and these facts, it strongly
suggests that a very close relationship lies be-
tween immunohistochemical localization of
GST and endogenous Px activity in endometri-
um of the rat uterus.

As endogenous Px activity, some researchers
suggested that it played a role in bacteriocidal
function, and others showed that it may play
a role in estrogen metabolism (11, 12, 20). Re-
cently, Metzler et al. (16) reported that en-
dogenous Px of the uterus specifically binds
DNA to estrogen in the presence of hydrogen
peroxide. Hydrogen peroxide seems to be the
agent responsible for initiating death in cells,
either directly or by initiation of lipid radical
formation (18, 19, 21). Furthermore, hydrogen
peroxide and superoxide are well known to in-
duce DNA damage (8, 9, 10). GST plays an
important role in detoxication by catalyzing the
conjugation of many hydrophobic and elec-
trophicil substances with reduced glutathione
(14). In addition, reduced glutathione may act
either by protecting cells from lipid peroxida-
tion due to secondarily formed superoxide radicals, or by protecting protein sulphydryls from
becoming irreversibly oxidized after oxidant in-
jury (7, 23, 24).

Based on our data and these facts, GST in
endometrium of the uterus strongly suggests
that it is an important defense system against
hydrogen peroxide and subsequent lipid peroxi-
dation.

It is well known that depletion of cellular
reduced glutathione, an important defense sys-
tem against oxidants, caused more rapid lysis
of the cells (2, 8, 22). In this respects, immu-
unocytochemical localization of enzymes in-
volved in glutathione metabolism including
glutathione peroxidase, glutathione reductase
and γ-glutamyl transeptidase must be holding
an important key to solve this problem, and
this is now under investigation in our labo-
atory.

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