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Protection from oxidative stress by superoxide dismutase in fertilization and early gestation

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I. INTRODUCTION

Reactive oxygen species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxy radical (OH:) possess potent oxygen toxicity to cells. Superoxide dismutase (SOD) is a metalloenzyme which is essential for the dismutation of O_2^- to H_2O_2 and O_2. SODs are important initial components in the cellular defense against oxygen toxicity, since O_2^- can react with H_2O_2 to generate single oxygen and hydroxy radicals that are even more reactive and cytotoxic than O_2^- or H_2O_2[1].

Active oxygen species such as superoxide are widely believed to play fundamental roles in many pathophysiological phenomena such as cellular aging and carcinogenesis[2-4].

In mammalian tissues, three superoxide dismutases (SOD); designated copper zinc SOD (Cu, Zn-SOD), manganese-SOD (Mn-SOD) and extracellular SOD exist[5-7].

The role of antioxidant enzymes is not clear in fertilization and early gestation. The role of superoxide anion in ovulation and luteal function was investigated by the localization of Cu, Zn-SOD and Mn-SOD in human ovary by immunohistochemical methods.

In addition, the role of antioxidant enzymes in human placenta was also studied to provide an insight to the antioxidant defense during gestation.

II. ROLE OF OXYGEN RADICAL AND SUPEROXIDE DISMUTASE IN HUMAN OVARY AND CORPUS LUTEUM

Eighteen ovarian specimens and fallopian tubes were removed from patients who had regular menstrual cycles and were to received simple or radical hysterectomy due to uterine myoma or cervical neoplasm. The ovarian specimens were removed from five patients in the early follicular phase, from six in the late follicular phase and from seven in the luteal phase. Four other ovarian specimens were removed from four patients with ectopic pregnancies. These specimens were rinsed immediately in a sterile saline solution and were deep frozen in liquid nitrogen until use. Written and verbal informed consent was obtained from all participants in an institution approved protocol according to the recommendations of the Declaration of Helsinki[8].

Granulosa cells were aspirated from 5 large follicles and 2 small follicles during IVF-ET program. These patients were stimulated with gonadotropin releasing hormone analog and human menopausal gonadotropin, and then were administered human chorionic gonadotropin at 36 hours before aspiration. These cells were mounted and dried on glass slides, and fixed by 4% paraformaldehyde immediately.

Immunohistochemistry was performed by the avidin-biotin-peroxidase system described for rat ovaries. Primary incubation was carried out with a 1:500 dilution of mouse lgG anti-human Mn-SOD[9], or with a 1:200 dilution of mouse lgG anti-human Cu, Zn-SOD[10].

Strong Mn-SOD reaction was found in the granulosa and theca cells of mature follicles and in granulosa cells that were aspirated in an IVF-ET program. However, a weak Cu, Zn-SOD reaction was found in the theca cells of mature follicles, but the aspirated granulosa cells were negative (Fig. 1). Menstural luteal cells showed moderate Mn-SOD immunoreactivity and weak Cu, Zn-SOD immunoreactivity. Strong Mn-SOD reaction was found in pregnant luteal cells, but a moderate Cu, Zn-SOD reaction was found only in the margin of the pregnant corpus luteum (Fig. 2). A summary of the immunohistochemical localizations of Cu, Zn-SOD and Mn-SOD in human ovaries, fallopian tubes and aspirated granulosa cells are shown in Table 1.

Mn-SOD was found in granulosa cells and theca cells of mature follicles, luteal cells of corpus luteum, and epithelial

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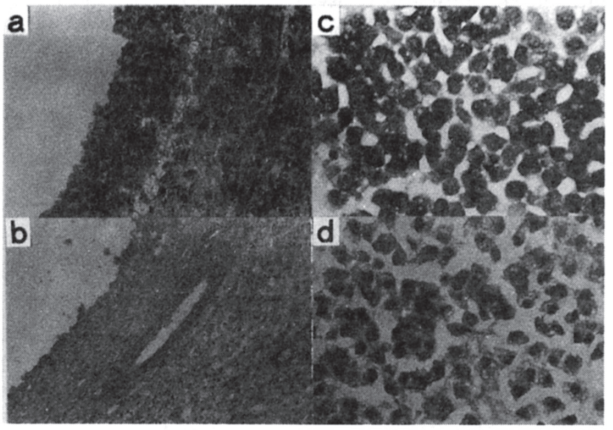

Fig. 1 Immunohistochemical localization of Mn-SOD (upper) and Cu, Zn-SOD (lower) in mature follicle and aspirated granulosa cells. Strong Mn-SOD was reaction found in granulosa cells and theca cells in mature follicle (panel a), but weak Cu, Zn-SOD reaction was found in theca cells (panel b). Magnification ×200. Strong Mn-SOD reaction was found in aspirated granulosa cells both in large follicles and small follicles (panel c). However, Cu, Zn-SOD reactivity was evident in these cells (panel d). Magnification ×400.

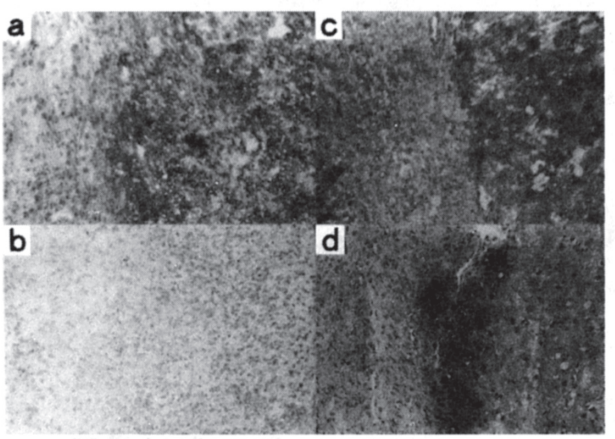

Fig. 2 Immunohistochemical localization of SODs in corpus luteum. Moderate Mn-SOD reaction was found in luteal cells that were obtained from patients in the postovulatoy phase (panel a). However, no Cu, Zn-SOD reactivity was evident in these cells (panel b). Magnification ×100.
Strong Mn-SOD reaction was found in luteal cells that were obtained from pregnant women in their 5th～8th gestational weeks (panel c). Moderate Cu, Zn-SOD reaction was found in only the margin of the corpus luteum which was taken at 5th and 6th gestational weeks of ectopic pregnancy (panel d). Magnification ×100.

cells of fallopian tubes. Cu, Zn-SOD was localized in theca cells of mature follicles, margin of corpus luteum and epithelial cells of tubal isthmus.

Hesla, et al. reported similar results for pseudopregnant rabbits [11]. The Mn-SOD in these cells may generate high levels of H_2O_2 to be made available for peroxidase action during

Table 1 Immunohistochemical localization of Cu,Zn-SOD and Mn-SOD in human ovary and aspirated granulosa cells

tissue type		cell type	Cu,Zn-SOD	Mn-SOD
ovary	mature follicles	granulosa cells	-	+～++
		thca cells	+	+～++
	corpus luteum	luteal cells (menstruationis)	-～+	+
		luteal cells (gravidiatais)	+	+～++
		granulosa cells (aspirated)	-	++

luteal steroidogenesis in the synthesis of progesterone [12]. Mn-SOD may be involved in oxygen toxicity in the production of steroids from active oxygen, or exhibit steroidogenesis activity in rat, rabbit and human ovaries.

Mn-SOD and Cu, Zn-SOD show different localizations and actions in the human ovary and fallopian tube, and the superoxide anion-SOD system may play an important role in ovulation and luteal function in human ovary.

III. SUPEROXIDE DISMUTASE IN GESTATION

1. Changes in Mn-SOD in maternal and fetal blood, and expression of Mn-SOD in placental fissue during pregnancy.

Figure 3 shows the change in Mn-SOD level of matermal blood at various gestational ages. Mn-SOD concentration in maternal blood decreased as gestation progressed (Fig. 3).

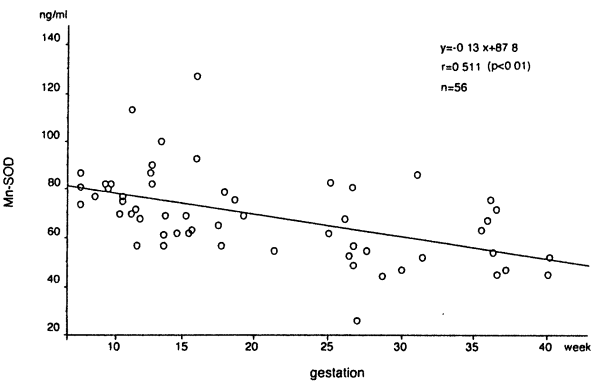

Fig. 3 Changes in Mn-SOD level of maternal blood at various gestational ages.

However, there was no significant change in Mn-SOD concentrations of umbilical blood during gestation. Also there was no significant change in Mn-SOD level in amniotic fluid during gestation.

Northern blot analysis was carried out using 20 μg of total RNA per placenta sample. A single 4Kb transcript of the Mn-SOD was present in the placental basal plate and villous tissue total cellular RNAs. Placental tissue showed two major bands,

1.0 and 4Kb, which could be due to variable splicing and the use of alternative polyadenylation signals as has been indicated in human Mn-SOD mRNA.

In the case of 19th gestational week, placental tissue contained elevated levels of message Mn-SOD mRNA compared to tissues of other gestational weeks (Fig. 4). The placenta

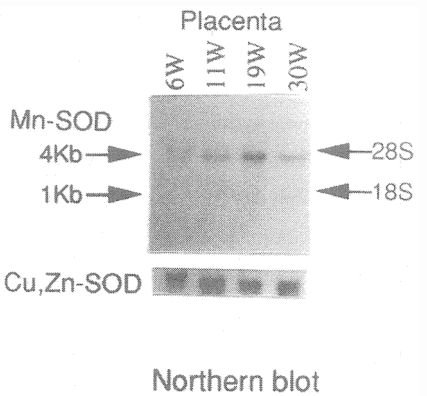

Fig. 4 Northern blot analysis of Mn-SOD in placenta.
Total RNA were prepared from placental tissues from 6th to 30th week gestation. Northern blot analysis was carried out using human Mn-SOD cDNA (upper panel) or human Cu, Zn-SOD cDNA (lower panel) as probes.

is one of the detoxification organs in the body that has a high metabolic rate as well as a rapid turnover of protein and enzymes.

In the present study, we confirmed that the Mn-SOD concentration of pregnant women decreased as gestation progressed, but it remained lower in the cord blood than in the maternal blood. We also found that Mn-SOD gene expression and its post-transcriptional processing are regulated with process of placental function until around the 20th week of gestation. Further definitive experiments will be required to understand the mechanism of Mn-SOD induction and whether or not this induction is linked to trophoblastic differentiation during gestation [13].

2. Effect of TNF on cultured human umbilical endothelial cells.

Since it is known that Mn-SOD is induced by TNF α in TNF-resistant cells to protect them from the cytotoxicity of TNF α, the expression of the mRNA for Mn-SOD and the enzyme level in endothellial cells were investigated.

Effect of TNF α on endothelial cells was examined. TNF α at concentration higher than 10 ng/ml moderately decreased the viability of EC. Since TNF α have both cytolytic and growth inhibitory effects, we examined LDH activity in the culture medium to assess whether the change in viability was due to cytolysis or to inhibition of growth. About 15% of the total LDH activity was found in the medium of EC incubated with TNF α at 24 hours. After 40 hours of incubation, TNF α induced a release from EC of about 30% of the total activity. Thus, the decreased viability of EC cells appeared to be due to the cytolytic effect of the cytokines employed (Table 2). Since it is known that Mn-SOD is induced by TNF α

Table 2 LDH Release from Human Vascular Endothelial Cells

Treatment	%LDH*	
	24hrs	40hrs
Endothelial cells		
Control	7.2 ± 0.5	17.4 ± 1.6
TNFα (10ng/ml)	15.1 ± 0.9	30.2 ± 0.8
IL-1α (100U/ml)	–	29.2 ± 1.0

Values are means ± SE ; n=3

*%LDH = (LDH in medium / [LDH in medium+ LDH in cell lysate]) x 100.

in TNF-resistant cells to protect them from the cytotoxicity of TNF α, the expression of the mRNA for Mn-SOD and the enzyme level in endothelial cells were investigated. As reported previously, Mn-SOD mRNA was about 40 − 50 folds by TNF α in endothelial cells. Expression of Cu, Zn-SOD, was not affected by these cytokines (Fig. 5).

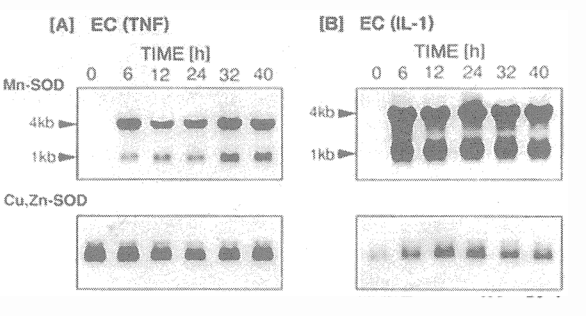

Fig. 5 Northern blot analysis of Mn-SOD mRNA after stimulation with TNF α and IL−1 α.
Total RNA were prepared from EC (A and B) cells following incubation with 100 ng/ml TNF α (A) or 50 ng.ml IL−1 α (B). Northern blot analysis was carried out using human Mn-SOD cDNA (upper panel) or human Cu, Zn-SOD cDNA (lower panel) as probes.

The Mn-SOD level within the cells was also measured by ELISA. An increase of Mn-SOD was observed in EC, which was concomitant with the expression of the mRNA. Since the

half-life of Mn-SOD in cells is relatively long, the level of this protein did not plateau even after 40 hours of incubation with these cytokines.

The level of Mn-SOD in conditioned medium of cells at various time intervals presence of TNF α were also determined. TNF α dramatically increased the levels of Mn-SOD in the culture medium of EC in a dose and time dependent manner.

The present study was undertaken to investigate the various effects of TNF α on human umbilical vascular endothelial cells. The increase in Mn-SOD in the culture medium of EC incubated with TNF α could be due to two indepencent functions of cytokine. One is the inducibility of Mn-SOD gene expression in EC probably through activation of certain protein kinases and transactivationg factors in the nucleus. The other is the cytolytic effect in which oxygen radicals generated from respiratory chain reactions in the mitochondria are involved [14].

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