Regional Distribution of Superoxide Dismutase Activity in Human Placenta and its Correlation with Lipid Peroxidation

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Abstract

The objectives of the present study are to determine the activity of superoxide dismutase (SOD) in various regions of human placenta, and to correlate this activity with lipid peroxidation. To follow up changes in SOD activity and lipid peroxidation during gestation, a female rat model was used and pregnant females were sacrificed at 4, 7, 10, 14, and 18 days of gestation. Placentas were collected from 45 uncomplicated term pregnancies. Each placenta was sampled in 3 regions on the maternal and fetal side: central; mid placenta; and periphery. 5 gm of tissue were obtained for analysis and full thickness samples were obtained from the umbilical cord. In the rat, 2-3 placentas from each (female (n=4) were pooled and assayed while the cord for all placentas of the same female were pooled and assayed. The results show slight regional variations in the amount of lipid peroxidation as estimated by malondialdehyde (MDA) with the mid placental region of the fetal side being significantly higher than any of the other placental regions. MDA concentration in the placenta and umbilical cord of female rat progressively increased with progress of gestation (67% increase in the placenta and 90% increase in the umbilical cord). The results also show slight regional variations in SOD activity in the placenta being higher at the fetal site. The SOD activity in the umbilical cord was significantly higher than any of the placental regions. SOD activity in the placenta and umbilical cord of female rat progressively increased with the progress of gestation and after 10 days SOD activity in the umbilical cord was significantly higher than the placenta. The results clearly show that the increase of lipid peroxidation in the placenta and umbilical cord during gestation is coupled with an increase in SOD activity. The magnitude of SOD activity increase is higher to compensate for the increase in lipid peroxidation.

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Keywords: placenta, umbilical cord, lipid peroxidation, SOD.

1. Introduction

Normal cells have a number of protective scavenger enzymes that act as antioxidants to detoxify the harmful reactive oxygen radical and prevent cell damage. One of these enzymes is superoxide dismutase (SOD: ECl.15.1.1) which catalyses the dismutation of superoxide radicals into oxygen and hydrogen peroxide and is widely distributed in mammalian tissues (McCord and Fridovich,1969; Bannister et al., 1987) including the placenta (Beckman, et al., 1973; Sekiba and Yoshioka, 1979; and Takehara et al, 1990). There are three SOD isoenzymes

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reports about the regional distribution of this enzyme in the placenta during gestation time. The objectives of this study are: (1) to measure the activity of the enzyme superoxide dismutase in various regions of human placenta immediately after delivery; (2) to determine the level of lipid peroxidation in the same placental regions; and (3) to correlate the level of lipid peroxidation with the activity of superoxide dismutase.

2. Materials and methods

2.1. Collection and processing of human placenta

Forty five placentas were collected from various maternity hospitals immediately after vaginal delivery and placed on ice. Each placenta was weighed and sampled in three main regions on both maternal and fetal sides as well as the umbilical cord as follows:

a- central region: within 3 cm of the cord insertion,
b- mid region: mid way between the cord insertion and margin,
c- peripheral region: within 3 cm of the placental edge, umbilical cord: full thickness tissue samples were obtained from the umbilical cord (midway between the placenta and the umbilicus).

At each sampling site 5g of tissue was obtained, split into a liquid phase and then kept in deep freeze (-80°C) until the time of processing.

2.2. Female rat model

Adult female rats weighing 250-300g were housed in groups of 4-5 in pvc cages (350x530 mm long x180 mm high) in an environment maintained at 19-22°C with 12/12 h light/dark cycle. Food and water were always available. Female rats were allowed to mate with male rats and the males were removed after pregnancy which was confirmed by the observation of vaginal plug. Pregnant females were sacrificed at various gestational age (4, 7, 10, 14, and 18 days).

Embryos were carefully removed, placenta and umbilical cords were separated and processed similar to human placenta as mentioned in section 2.1. Each 2-3 placentas were pooled and assayed, but the cord of all placentas of the same female was sampled and assayed.

2.3. SOD assay

Placental tissues were homogenized in phosphate buffered saline (PBS, PH 7.4), sonicated and then centrifuged at 10,000 g for 25 min to obtain the cytosol fraction which was used to assay for Cu/Zn SOD. The assay was done according to the method of Misra and Fridovich (1972) at 30°C. The amount of enzyme that causes 50% inhibition of epinephrine auto-oxidation is defined as 1 unit.

2.4. Measurement of lipid peroxidation

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) (Buege and Aust, 1978). The MDA level was measured in the cytosol fraction of placental homogenate. Lipid peroxides were measured after addition of 2ml of TBA reagent (15% w/v trichloroacetic acid and 0.25 N HCl) to 1 ml of cytosol fraction. The mixture was treated in a boiling water bath for 15 minutes. After cooling, the suspension was centrifuged at 1000 g for 10 minutes. The supernate was then separated and absorbance was measured at 535 nm. The MDA concentration was determined by the specific absorbance coefficient (1.34x105 mol/cm3).

2.5. Measurement of protein

Protein concentration in the cytosol suspension was determined by a modification of the Lowry procedure (1951) as reported by Markwell et al. (1978) using bovine serum albumin as a standard.

2.6. Statistical methods

Statistical analysis was done using a t-test (SPSS for windows) by a personal computer. P values <0.05 were considered significant.

3. Results

3.1. Topographical data of maternal and placental records

The placental tissue was collected from 45 uncomplicated term pregnancies (38-40 weeks menstrual age). Mean weight of placenta was 720g (range 610-945 g). Medical records for the women from whom placentas were recovered revealed that the mean maternal age was 28.4 years (range 19-38 years), mean gestational age was 38.6 weeks (range 37-42 weeks), mean parity was 3.7 (range 0-7), and mean birth weight of infants was 3028 g (range 2100-4030 g). Each placenta was sampled in 3 main regions on both the maternal and fetal sides. The various regions in the placenta which were dissected are indicated in Figure 1.

3.2. Regional distribution of SOD activity in the placenta

3.2.1. Regional distribution of SOD in human placenta

Distribution of SOD activity in various regions of the placenta and the umbilical cord are shown in Table 1. There are slight regional variations in the SOD activity being relatively higher at the fetal site. There is no significant difference in SOD activity of various regions at the same site of the placenta. However, the central region of the fetal site has a significantly higher level of SOD (P < 0.05) than any of the regions in the maternal site. Activity of SOD was significantly higher (P < 0.01) in the umbilical cord than any of the regions in either the maternal or fetal sites of the placenta.

3.2.2. SOD activity in placenta of female rat

Because the placenta of the rat embryo is much smaller than human placenta and the weight of each placenta was less than 0.5 g, regions of the placenta were not dissected. Two or three placentas were pooled and processed for SOD activity. Changes in the SOD activity, in the placenta...
and the umbilical cord are shown in Table 2. SOD activity in either the placenta or the umbilical cord, progressively increased with the progression of gestational time. Until 7 days of gestation, there was no significant difference in SOD activity in the placenta compared to the umbilical cord. Although the activity of the enzyme in the placenta or the umbilical cord showed slight progressive increase, such increase was not significant. At 10 days of gestation, SOD activity in the placenta increased 58% while in the umbilical cord it increased about 2 folds. The increase in SOD activity in the placenta and the umbilical cord persisted throughout the rest of the gestational period. However, the increase was more evident in the umbilical cord. At 18 days, SOD activity in the placenta reached 2.1 folds, while in the umbilical cord it reached 3.1 folds.

3.3 Lipid peroxidation in the placenta

3.3.1 Lipid peroxidation in human placenta

Lipid peroxidation in various placental regions is shown in Table 3. The average MDA concentration in the maternal side was 1.42 nmol/mg (range 1.41 – 1.53). In spite of the slight regional variation, there was no significant difference (P>0.05). The average MDA concentration in the fetal side was 1.72 nmol/mg (range 1.62 – 1.83).

There are slight regional variations in the MDA concentration being relatively higher at the mid placental region. There is no significant difference in MDA concentration of various regions at the same site of the placenta.

However, the mid placental region of the fetal site was significantly higher (P < 0.05) than any of the regions at the maternal site. Also the average MDA concentration in the fetal site was significantly higher (P < 0.05) than the average maternal site. The MDA concentration in the umbilical cord was significantly higher than either the maternal or the fetal site of the placenta (P<0.05). It was 56% higher than the maternal site and 29% higher than the fetal site.
Table 1: Regional distribution of SOD activity in human placenta and umbilical cord

<table>
<thead>
<tr>
<th>1. Placenta</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Maternal Side</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. M1 (Central)</td>
<td>3.31 + 0.41</td>
<td>3.31 + 0.41</td>
</tr>
<tr>
<td>b. M2 (Mid placenta)</td>
<td>3.36 + 0.39</td>
<td>3.36 + 0.39</td>
</tr>
<tr>
<td>c. M3 (Periphery)</td>
<td>3.42 + 0.45</td>
<td>3.42 + 0.45</td>
</tr>
<tr>
<td>B. Fetal side</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. F1 (central)</td>
<td>4.21 + 0.6*</td>
<td>4.21 + 0.6*</td>
</tr>
<tr>
<td>b. F2 Mid placenta</td>
<td>4.13 + 0.53</td>
<td>4.13 + 0.53</td>
</tr>
<tr>
<td>c. F3 (Periphery)</td>
<td>3.82 + 0.45</td>
<td>3.82 + 0.45</td>
</tr>
<tr>
<td>2. Umbilical cord</td>
<td>5.8 + 0.73**</td>
<td>5.8 + 0.73**</td>
</tr>
</tbody>
</table>

N = 45, Values are mean ± S.D, * P < 0.05 compared to maternal side, ** P < 0.01 Compared to placental region

Table 2: Changes of SOD activity in placenta and umbilical cord of female rat during gestation

<table>
<thead>
<tr>
<th>Gestation Age (days)</th>
<th>Fetus weight (g)</th>
<th>Placenta weight (g)</th>
<th>SOD activity (unit/mg protein) Placenta</th>
<th>SOD activity (unit/mg protein) Umbilical cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.4 + 0.2</td>
<td>0.23 + .02</td>
<td>2.6 + 0.3</td>
<td>2.5 + 0.4</td>
</tr>
<tr>
<td>7</td>
<td>2.1 + 0.2</td>
<td>0.31 + .03</td>
<td>2.7 + 0.4</td>
<td>3.1 + 0.5</td>
</tr>
<tr>
<td>10</td>
<td>3.1 + 0.3</td>
<td>0.35 + .03</td>
<td>4.1 + 0.4*</td>
<td>5.2 + .07*</td>
</tr>
<tr>
<td>14</td>
<td>3.6 + 0.4</td>
<td>0.38 + .04</td>
<td>4.6 + 0.5*</td>
<td>5.7 + .08*</td>
</tr>
<tr>
<td>18</td>
<td>4.4 + 0.5</td>
<td>0.45 + .05</td>
<td>5.5 + 0.6**</td>
<td>7.8 + .09**</td>
</tr>
</tbody>
</table>

N = 4 female rats, 2-3 placentas were pooled from each female rat and umbilical cords of all placentas of the same female were pooled. values are mean ± S.D, * P < 0.05 , ** P < 0.01

Table 3. Lipid peroxidation in various regions of human placenta as estimated by MDA
3.3.2. Lipid peroxidation in the placenta of female rat

Lipid peroxidation in the placenta of female rat is shown in Table 4. MDA concentration in the placenta and the umbilical cord progressively increased with the progression of gestational time. Until 10 days of gestation, there was no significant difference in lipid peroxidation between the placenta and umbilical cord. At 14 and 18 days the difference in MDA concentration between the placenta and the umbilical cord was significant (P < 0.05), with the umbilical cord being higher.

MDA concentration in the placenta increased 67% at 18 days while in the umbilical cord it increased 90%.

### Type of Tissue | MDA concentration (nmol/ mg protein X 10^-1) 
---|---
1. Placenta |  
A. Maternal side |  
a. M1 (central) | 1.41 ± 0.16 
b. M2 (mid placental) | 1.32 ± 0.14 
c. M3 (peripheral) | 1.53 ± 0.16 
B. Fetal side |  
a. F1 (central) | 1.62 ± 0.18 
b. F2 (mid placenta) | 1.83 ± 0.21* 
c. F3 (peripheral) | 1.71 ± 0.19 
2. Umbilical cord | 2.21 ± 0.23**

N=45, values are mean ± S.D , *P< 0.05 compared to maternal side. **P<0.01 compared to placental region.

4. Discussion

The current study determined the level of lipid peroxidation in human and female rat placentas. We found that the level of peroxidation in both the human and the rat placenta are comparable. Concentration of peroxidation as estimated by MDA in the placenta of female rat at 18 days (2 days before delivery) was higher than that in full term delivered human placenta. The level was 2.45 nmol/mg in the rat versus 1.5 nmol/mg (average of the maternal and fetal regions) in humans (Table 3 and 4). The level is 63% higher in female rat.

The placenta is a heterogenous tissue with membraneous nature (Ali et al., 1996); so different tissue compartments probably contributed to the tissue level of lipid peroxides (Rojimakers et al., 2004). This could explain the slight variation in the level of peroxidation at various regions of the placenta being relatively higher at the fetal site. In both human and female rat, the level of lipid peroxidation in the umbilical cord was significantly higher than the placenta. In human placenta, the level of peroxidation in the umbilical cord was 47% higher than the placenta while in the female rat the level of peroxidation was about 12% higher. This indicates that the umbilical cord has a considerable contribution to the formation of lipid peroxides.

The level of peroxidation in the placenta or the umbilical cord, as shown in the present study, is high compared to other tissues like liver or lung as reported by Beckman et al., 1973; Romero et al., 1998; Janssen and Tazzero, 2002). This high level could be due to increased thromboxane production as reported by various investigators (Wang et al., 1992; Walsh and Wang, 1995; Cueto et al., 1997), which causes an increase in cylooxygenase activity resulting in increased oxygen radical formation. Lipid peroxides are formed when oxygen radicals interact with polyunsaturated fatty acids. Since the placenta is a rich source of unsaturated fatty acids, the increased cylooxygenase activity associated with increased thromboxane production could be coupled with increased placental peroxidation.

In this study, we also shown that lipid peroxidation progressively increases as gestation progress in both the placenta and the umbilical cord. The increase was 67% in
Table 4. Lipid peroxidation in the placenta and umbilical cord of female rat during gestation as estimated by MDA

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<th>Type of Tissue</th>
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N=45, values are mean ± S.D., *P<0.05 compared to maternal side. **P<0.01 compared to placental region

The placenta and almost doubled in the umbilical cord (Table 4). Although several investigators (Sekiba and Yoshika, 1979; Wickens, 1981; Wang et al., 1992; Wang and Rogers, 1997) have also shown increased level of lipid peroxides during pregnancy (review Walch, 1994) this was in maternal blood and not in actual placental tissue as we have demonstrated. It is also worth mentioning that only scarce reports (Palan et al., 2001) are available about lipid peroxidation in the umbilical cord compared to the placenta.

We have shown in a previous study that the walls of blood vessels do have considerable amount of lipid peroxidation and this is much higher in the varicose veins (Wali et al., 2002).

In this study, we have also shown that SOD activity in the placenta exhibited slight regional variations being higher at the fetal site of the placenta, particularly the central region (Table 1). The SOD activity was significantly higher in the umbilical cord than any of the placental regions of either the maternal or the fetal site. The level of SOD in the placenta of female rat at 18 days of gestation was 48% higher than in the human placenta (5.5 units/mg in the female rat versus 3.71 units/mg in human).

The level of SOD activity in the umbilical cord of female rat was 35% higher than that in humans.

There was a progressive increase of SOD activity with progress gestation where the increase was 2 folds in the placenta and 3 folds in the umbilical cord (Table 2).

Results in this study clearly show that the increase of lipid peroxidation in the placenta and the umbilical cord during gestation is coupled with an increase in SOD activity. The magnitude of the increase in SOD activity is higher than the increase of lipid peroxidation in female rat. Correlation of the increase in lipid peroxidation and the increase in SOD activity is shown in Figure 2. This indicates that although lipid peroxidation increased with the progress of gestation, the antioxidant SOD activity has also increased to compensate for the increase in lipid peroxidation.
Various investigators have reported the increase of lipid peroxidation during pregnancy (Diamant et al., 1980; Hubel et al., 1989; Walsh, 1994) which seems to play an important factor in the pathogenesis of pre-eclampsia.

Recently, there has been a growing interest in using antioxidants to suppress lipid peroxidation in various tissues including the placenta (Chow, 2001; Raijmakers, et al., 2004). In a previous study, we have shown that treatment with vitamin E considerably reduced lipid peroxidation in spermatozoa and improved sperm motility. Therefore, supplementation with vitamin E could protect against the increase of lipid peroxidation in the placenta.

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