Experimental Study on the Effects of Acute Carbon Monoxide Intoxication during Pregnancy on Fetal Growth in Rat

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Abstract: This study was carried out to observe the effect of acute carbon monoxide (CO) poisoning on the gestational process and outcome, and to develop the proper method to evaluate it. Primiparous Sprague-Dawley rats were exposed for 1 hour to a CO of 1800 ppm or 2 hours to a CO of 1400 ppm resulting in blood carboxyhemoglobin levels of 68-72% on day 11 of gestation when active organogenesis is beginning. Half the rats exposed to a CO of 1400 ppm for 2 hours were treated with hyperbaric oxygen (HBO) 15 minutes after CO exposure.

Only the group exposed to a CO of 1400 ppm for 2 hours sustained significant damage to the pregnancy. Though no significant pregnancy interruption resulted in the group exposed to CO at a concentration of 1800 ppm for 1 hour, the fetal growth were significantly retarded. The fetuses of rats treated with HBO after exposure to CO did not show growth retardation other than a decrease in the ratio of biparietal diameter to crown-rump length. Carbon monoxide was not found to be teratogenic in any experimental groups.

Keywords: Carbon monoxide poisoning, Hyperbaric oxygenation, Fetal growth, Malformation, Pregnancy wastage

INTRODUCTION

Carbon monoxide (CO) is produced by the incomplete combustion of organic materials. Carbon monoxide is one of the oldest and most common agents of poisoning; it has been with us since humans first used fire for warmth, cooking and defense. CO poisoning is uniquely associated with the history of civilization.

In Korea, a very unique underfloor heating system called Ondol is routinely used and further coal mostly in the form of coal briquettes is used as the main space heating source for 56.4% of total housing units (NBS, EPB 1982). These system produce CO and then CO poisoning has been regarded as one of the most serious public health problems (Bin 1966; Sohn 1967; Hwang 1969; Kim et al. 1972; Yun and Cho 1977; Kim et al. 1980; Cho et al. 1985; Cho et al. 1986).

According to the results of epidemiological surveys, each year in Korea, an estimated one million persons are affected by CO intoxication at the incidence rate ranging from 200 to 400 per 10,000 persons at risk; at least 3,000 persons die yearly from accidental exposure to high concentration of CO, and more than 140,000 persons receive emergency care because of altered mental state (Yun and Cho 1977; Cho et al. 1985). Females were more commonly affected than males with 45% of the female cases in their child-bearing years (Cho et al. 1985).

Pregnant women are at increased risk for CO poisoning. The pregnant woman has increased endogenous production of CO, production of CO by developing fetus, an increase of resting respiratory minute volume, and a tendency to anemia (Smith et al. 1935; Shephard 1983).
Since the first report in 1859 of CO intoxication in pregnant women, more than 50 cases have been reported (Freun: 1859; Tissier 1909; Niclou 1913, Philips 1924; Müller and Graham 1955; Goldstein 1965; Cramer 1982). Review of these cases shows that the evidence for CO poisoning of the mother and the effect on the fetus varies greatly. Müller and Graham (1955), Tedeschi (1956), Ingalls and Philbrook (1958), Schwedenberg (1959), Chu and Chi (1980), and Chi and Park (1983) reported some cases of hydrocephalus, microcephalus, exencephalus, ectromelia and other malformations from neonatal or intrauterine deaths of a full-term fetus due to CO poisoning.

Since the first experiment in rabbits in 1877 by Hogyes, workers have exposed pregnant animals to CO to explain the detrimental effect of CO on pregnancy. In reproduction studies, Williams and Smith (1935), Lee et al. (1974), Moon and Cha (1976), Lim et al. (1977), Cho et al. (1978) found that acute or chronic CO poisoning induced by exposure of albino rats to a sublethal concentration of CO resulted in a significant increase of fetal absorption and death. In a few studies of the possible effect of CO on embryonal and fetal development of rats, Lee et al. (1974) and Choi and Oh (1975) presented some fetal malformations with club-foot, microphthalmia or ectromelia. However, Cho et al. (1978) could not find the teratogenic effect of CO exposing rats for 15 minutes to 4500 ppm CO.

There are many physiological and anatomic similarities between rodents and humans that justify use of the rat in toxicological evaluations. However, in teratology and transplacental toxicology, aspects of reproduction that distinguish rodents from humans must be considered (Oser 1981). Differences in uterine architecture may play such a critical role in interspecies comparisons that evaluation of effects on pregnancy from the maternal aspect would be different that the fetal aspects. The purpose of the present study is to determine whether acute exposure of pregnant rats to CO would adversely alter the course of embryonal or fetal development and to elucidate the feasibility for some parameters of the fetus in evaluating the influence of CO on pregnancy. This study also evaluates the role of hyperbaric oxygenation in reducing the effect of CO on pregnancy.

**MATERIALS AND METHODS**

**Experimental animals**

Female virgin albino Sprague-Dawley rats weighing 70-90 g were housed in groups of four to six in cages under the same environmental conditions. They were fed a commercial pelleted laboratory ration and tap water ad libitum. After 6-8 weeks of breeding, rats with a weight ranging from 190 to 220 g were mated overnight. Mating was confirmed by detection of vaginal plug or spermatozoa in the vaginal smear the following morning (day 1 of gestation). Mated females then were separated from the males and assigned randomly to treatment or control groups.

**Animal exposures**

On day 11 of gestation when active organogenesis starts, the pregnant rats were exposed to CO in an air-tight exposure chamber measuring 30 cm in diameter and 60 cm in length. Precalibrated gas mixtures (Korea Standards Research Institute) that contained either 1800 ppm or 1400 ppm CO in ambient air were employed. The duration of CC exposures were one hour (h) in a concentration of 1800 ppm and 2 h in 1400 ppm. Flow rate through each exposure was approximately 10-15 l/min to ensure rapid removal of waste gases. Carbon monoxide was continuously monitored with a CO analyzer (Gastec, CM-525 HB). Exposure to CC at these conditions resulted in loss of righting reflex and coma after 53-60 minutes exposure to 1800 ppm CO or 98-120 minutes exposure to 1400 ppm CO with spontaneous recovery in less than 4-15 minutes. Fatality rates were 0% and 3%, respectively.

Control rats were maintained in the same room in a similar chamber. Except for no inhalation of CO, their conditions were identical to those exposed to CO.

Half the rats exposed to 1400 ppm CO were treated with hyperbaric oxygen 15 minutes after exposure. In an acryl-made chamber (25 cm in diameter, 74 cm in length and 1 cm in thickness; they were kept at 3 atmosphere absolute (ATA); 100% oxygen for 45 minutes.

**Carboxyhemoglobin**

Carboxyhemoglobin (COHb) levels were determined on blood from non-pregnant rats to avoid unnecessary influences on pregnancy by taking blood sample. Blood sample were taken from cardiac puncture 5 minutes, 12 minutes, 20 minutes,
minutes and 60 minutes after the CO exposure measured with IL-282 CO-Oximeter.

**Observations**

II of the mated rats were observed daily, shed during the experimental period, and were delivered by cervical dislocation on day 21, the day before expected parturition. The uterus was resected from each rat and the position and the number of live, dead, and resorbed fetuses were recorded. After being weighed, live fetuses were fixed in Bouin’s solution for about 2 weeks. After dehydration, the fetuses were thoroughly rinsed with water and were measured for crown-rump length (CRL), biparietal diameter (BPD), tail length (TL), and limb lengths. All the fetuses were examined for external alterations in a systemic manner from head to tail with the naked eye or under a dissecting microscope.

After examining the external features, all fetuses were then cut in slices according to the method described by Wilson and Warkany (1965) with razor blades in 1-2 mm thick sections to observe the internal malformations under the dissecting microscope.

**RESULTS**

The blood level of COHb immediately after exposure to 1400 ppm CO for 2 h was 68.1% and increased in a single exponential curve. The reported half-clearance time of CO elimination was estimated as about 28 minutes for rats breathing ambient air and about 17 minutes for rats treated with hyperbaric oxygenation (Fig. 1).

The mean maternal weight gains during pregnancy are presented in Table 1. Slight, but insignificant decrease in weight gain were observed in Group I, rats receiving 1800 ppm CO for 1 h. Group IV, rats receiving hyperbaric oxygenation or exposure to 1400 ppm CO for 2 h. However, significant differences in maternal body weight gain were evident between Group III, rats receiving 1400 ppm CO for 2 h and controls. From the data on the daily number of rats observed. No dead fetus was observed at any groups. P.I.R. for control (Group I), Group II, Group III, and Group IV was 15.4%, 3%, 52.9% and 28.6%, respectively. Only the Group III, females exposed to 1400 ppm CO for 2 h showed a significant increase in P.I.R. Exposure of pregnant female rats to CO for 1 or 2 h did not significantly reduce litter size. In 13 control litters, the mean number of pups was 10.9 (range, 8 to 14). In 18 litters exposed 1 h to 1800 ppm CO, the number of pups was 12.4 (range, 8 to 17). In 17 litters exposed 2 h to 1400 ppm CO, the mean number of pups was 10.6 (range, 8 to 13). In 14 litters treated with HBO after exposure to 1400 ppm CO for 2 h was 10.5 (range, 7 to 13). From this finding, it seems that live-litter size is not a sensitive indicator in evaluating the effect of CO on pregnancy. Carbon monoxide intoxication on day 11 of gestation decreased embryo survival, as reflected in an increase in fetal mortality rate (Table 2). However, among three experimental groups, only the group III, rats receiving 1400 ppm CO for 2 h showed significant increase.

As physical parameters, fetal weight, biparietal diameter (BPD), crown rump length (CRL), forelimb length (FLL), hindlimb length (HLL), and tail length (TL) were used to denote generalized or localized retardation of fetal growth. Using these parameters, even exposure to 1800 ppm CO for 1 h caused significant retardation of general fetal growth in spite of negative findings on pregnancy wastage determined by the variables related to the maternal side (Table 2). Therefore, the values of physical parameters of Group IV got closer to that of control than Group II and Group III (Fig. 2).

Throughout the examination on the external and
Table 1. Effect of carbon monoxide intoxication at day 11 of gestation on maternal weight gain, resorptions and litter size

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of females</td>
<td>13</td>
<td>18</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Average weight gain during pregnancy</td>
<td>56.3±10.16</td>
<td>50.9±8.19</td>
<td>48.8±7.34</td>
<td>52.1±10.91</td>
</tr>
<tr>
<td>Number of rats with resorptions</td>
<td>2</td>
<td>6</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Pregnancy interruption rate</td>
<td>15.4%</td>
<td>33.3%</td>
<td>52.9%*</td>
<td>15.6%</td>
</tr>
<tr>
<td>Average number of live fetuses per dam</td>
<td>10.9±1.72</td>
<td>12.4±2.55</td>
<td>10.6±1.50</td>
<td>10.5±1.74</td>
</tr>
</tbody>
</table>

*Significantly different from the control value, p < 0.05

1 Group I: control, Group II: rats receiving 1800 ppm CO for 1 h, Group III: rats receiving 1400 ppm CO for 2 h, Group IV: rats treated with hyperbaric oxygenation after exposure to 1400 ppm CO for 2 h.
2. Per cent of weight gain at term to the weight at day 1 of gestation, mean±S.D.
3. Pregnancy Interruption Rate (P.I.R.)

\[
\text{P.I.R.} = \frac{\text{Number of rats with resorptions}}{\text{Number of females}} \times 100
\]

Table 2. Effect of carbon monoxide intoxication at day 11 of gestation on resorptions and physical parameters of experimental and control fetuses

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of implants</td>
<td>144</td>
<td>231</td>
<td>195</td>
<td>153</td>
</tr>
<tr>
<td>Number of resorptions</td>
<td>3</td>
<td>8</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Number of fetal deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fetal mortality rate (%)</td>
<td>2.08</td>
<td>3.46</td>
<td>7.69*</td>
<td>3.27</td>
</tr>
<tr>
<td>Number of fetuses measured</td>
<td>141</td>
<td>223</td>
<td>180</td>
<td>148</td>
</tr>
<tr>
<td>Fetal weight (gm)</td>
<td>3.69±0.37</td>
<td>3.39±0.41</td>
<td>3.40±0.42</td>
<td>3.60±0.36</td>
</tr>
<tr>
<td>Biparietal diameter (BPD, mm)</td>
<td>9.17±0.58</td>
<td>8.92±0.53</td>
<td>8.77±0.89</td>
<td>9.19±0.64</td>
</tr>
<tr>
<td>Crown-rump length (CRL, mm)</td>
<td>33.24±3.06</td>
<td>32.65±1.89</td>
<td>32.63±2.76</td>
<td>33.65±1.47</td>
</tr>
<tr>
<td>Forelimb length (FLL, mm)</td>
<td>9.49±0.70</td>
<td>9.26±0.71</td>
<td>9.22±0.78</td>
<td>9.60±0.61</td>
</tr>
<tr>
<td>Hindlimb length (HLL, mm)</td>
<td>6.30±0.92</td>
<td>6.09±0.85</td>
<td>6.27±0.69</td>
<td>6.43±0.83</td>
</tr>
<tr>
<td>Tail length (TL, mm)</td>
<td>14.25±0.85</td>
<td>13.54±1.12</td>
<td>14.04±1.05</td>
<td>14.31±0.86</td>
</tr>
<tr>
<td>BPD/CRL ratio</td>
<td>0.280±0.05</td>
<td>0.274±0.02</td>
<td>.272±0.04</td>
<td>0.274±0.02</td>
</tr>
</tbody>
</table>

*Significantly different from the control value, 0.01 < p < 0.05
**Significantly different from the control value, p < 0.01
1. Fetal mortality rate

\[
\text{Fetal mortality rate} = \frac{\text{Number of resorptions and fetal deaths}}{\text{Number of implants}} \times 100
\]
the internal malformations, none of the fetus with malformation was found.

**DISCUSSION**

Since the work of Claude Bernard in 1857 first proved that CO combined with hemoglobin to form carboxyhemoglobin, the pathophysiology of CO poisoning has been understood. The mechanism of CO poisoning included: inhibition of oxygen transport, reduction of oxygen delivery to the tissues, and impairment of oxygen utilization by the tissues. Carbon monoxide gives rise to anemic hypoxia by virtue of an affinity for the ferrous heme of hemoglobin that is about 250 times greater than that of oxygen. This shifts the dissociation curve of oxyhemoglobin to the left so that less oxygen is available to the tissues at a particular oxygen tension (Roughton and Darling 1944). The affinity of CO for the iron of cytochrome oxidase implies the possibility of an additional histotoxic hypoxia (Brieley 1976).

Many authors have discussed the harmful effects of CO exposure on fetal development (Wells 1933; Williams and Smith 1935; Goldstein 1965; MacMahon et al. 1965; Fechter and Annau 1977) and perinatal mortality (Longo 1977). Experiments by Curtis et al. (1955) and clinical observations done by Freiberg et al. (1959), Gemzell et al. (1959), Goldstein (1965) and others, have demonstrated that direct CO poisoning of the fetus occurs when its carrier is exposed to CO.

There are some differences between mother and fetus in the mode of distribution of CO. A transient CO exposure may yield lower peak COHb readings in the fetus than in the mother, however, the fetus sustains 10 to 25% higher twenty-four-hour average COHb readings (Longo 1970; Hill et al. 1977). The fraction of CO that is absorbed by the mother penetrates to the fetus unless the exposure is so severe that the mother is herself killed very quickly. Fetal uptake of CO occurs two to three times more slowly in the fetus than in the mother, and is eliminated slowly from the fetus as well (Hill et al. 1977). Harm may result not only from the impairment of oxygen transport within the fetus but also from a derangement of placental metabolism. The acute effects of CO exposure can thus have a disastrous effect upon the unborn child.

While fetal hypoxia is most likely in the final trimester of pregnancy, disturbances of fetal growth are more probable if fetal oxygenation is impaired during the first trimester. The critical period for CO poisoning has been reported as the 11th day of gestation in rats (Wilson 1953; Cho and Yun 1982). Day 11 of gestation in rats is compatible with the 17th weeks of Witches’s Standard Stage (Altman and Ditter 1972; Schneider and Norton 1972), at which active organogenesis starts.

The harmful effects of CO poisoning depend upon variables in the hosts environment even though a given carboxyhemoglobin reading reaches the same value. In this experiment, there is a little difference in peak COHb level between the rats receiving 1800 ppm CO for 1 h and those exposed to 1400 ppm CO for 2 h. In evaluating pregnancy wastage by weight gain during pregnancy, pregnancy interruption rate or fetal mortality rate, rats exposed to 1400 ppm CO for 2 h showed more prominent pregnancy wastage rather than those receiving 1800 ppm CO for 1 h. However, when pregnancy wastage was evaluated with fetal growth, rats receiving 1800 ppm CO for 1 h also showed significant retardation compared to the control.

In the field of teratogenic and transplacental toxicology, ethical considerations have precluded the deliberate administration of CO to pregnant woman.
The available evidence is thus limited to animal experiments and occasional accidental exposure. The rat is a good animal in which to perform CO exposure experiments, as it attributes included: a short gestational period, similar affinity for CO of the hemoglobin to man and relative susceptibility to CO (Fodor and Winneke 1971; Alexandrov 1973). Differences in architecture of the uterus, however, play a critical role in interspecies comparisons. Therefore, it is important to define the criteria adopted for evaluation prior to make a conclusion concerned to polytocousous animals.

Carbon monoxide is eliminated almost entirely via the lungs in an exponential manner. Under resting conditions, the resultant half-time of CO elimination in human is generally accepted as about 3 to 4 h for a subject breathing air and about 1 h breathing oxygen at normal atmospheric pressure (Pace et al. 1950; Bartlett 1968); somewhat faster rates are observed at very high COHb concentrations and slower rates at very low concentrations. The elimination half-times vary somewhat as a function of initial COHb concentration. Factors involved in the elimination of CO are the amounts of CO and oxygen present, the magnitude of ventilation, age, sex (Pace et al. 1950; Rode et al. 1972; Peterson and Stewart 1975), the status of consciousness, health status (Britten and Myers 1985), and altitude of the patient (Myfre 1970). In experimental animals, the elimination of CO is dependent on the species and body mass of the animal (Tyuma et al. 1981).

Since hyperbaric oxygenation was introduced by Haldane in 1995 for the treatment of CO poisoning, it has been regarded as the best choice of treatment for CO poisoning, especially for acute exposure to high concentration of CO. With HBO, carbon monoxide is eliminated much more rapidly from the hemoglobin and also from cytochrome oxidase, in accordance with the law of mass-action, which drives the equation to the left (Smith 1963). If a patient is allowed to breathe at 3 ATA, the half time for elimination of COHb is reduced to 23 minutes (Pace et al. 1950); elimination of COHb for the cytochrome oxidase should be increased so that the condition of histotoxic hypoxia could be corrected. In this experiment, the dissociation curve of COHb for rats showed a single exponential curve. The effect of HBO on the elimination of CO was measured by way of half-clearance time of COHb. As the estimated blood level of COHb (46%), at 15 minutes after CO exposure to 1400 ppm CO for 2 h was used as a initial level to be compared, half-clearance time for rats treated with HBO was shortened as 1/4 compared to rats breathing ambient air. Consequently, the protective effect of HBO that no significant maternal or fetal variables during CO inhalation at day 11 of gestation was similar to those reported by Cho and Yun (1982) and by Cho (1983).

Fetal damage, not death, has been reported repeatedly after accidental or suicidal maternal exposure to illuminating gas or other sources of CO. In a female mentioned by Ingalls and Philbrook (1958), the extremities were grossly deformed except for a normal right arm: the mother's pregnancy had been complicated by an episode of severe CO poisoning at 5-7 weeks of gestation. Chu and Chi (1980) reported one case with exencephalus from a 26-year-old female who had a history of CO poisoning during the early gestation period.

Despite several case reports on the teratogenic effects of maternal CO intoxication, not many animal studies have been done. Choi and Oh (1975) exposed pregnant rats to 750 ppm CO for 3 h during from 7 to 9 day of gestation. This resulted in 37.5% fetal mortality rate and 4.14% rate of fetus malformations in the exposed group, as contrasted with 2.5% and 2.56% in the control group, respectively. Lee et al. (1974) exposed rats to coal briquette gas contained 1500 ppm CO and 5 ppm SO2 for 30 minutes daily during 21 day pregnancy. Among 69 pups from 10 females, 2 fetuses with ectromelia were observed. In another group exposed to relatively low concentration of briquette gas (750 ppm CO and 2.5 ppm SO2), 1 of 113 fetuses showed malformations with ectromelia as contrasted with none of malformation in the control group. However, Cho et al. (1978) did not find any malformations among the fetuses of rats exposed to 4500 ppm CO for 15 minutes at day 6 or 13 of gestation. Schwetz et al. (1979) exposed mice to CO at a concentration of 250 ppm for 7 or 24 h daily during the period of major organogenesis, days 6 through 15 of gestation. As a result, exposure for either 7 or 24 h daily to 250 ppm was not teratogenic, but a small number of malformed fetuses were observed in the experimental groups at an incidence which was not statistically significantly greater than in the control group. The results of this experiment indicate the absence of a teratogenic effect on rats which inhaled CO at a con-
centration either 1400 ppm for 2 h or 1800 ppm for 1 h during the early period of organogenesis.

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임신 중 급성일산화탄소중독이 백서태자의 성장에 미치는 영향에 관한 실험적 연구

서울대학교 의과대학 예방의학교실
조수현 · 이수동 · 윤덕로

일산화탄소 발생량이 많은 염탄을 주 남방염로로 사용하고 있는 우리나라에서는 일산화탄소 중독의 위험이 가장 심각한 보편문제중 하나로, 이러한 중독환자중에는 생리적으로 일산화탄소에 대한 감수성이 높은 일부도 상당수 포함되어 있을 것으로 생각되어 일부에 있어서의 일산화탄소중독, 그리고 이의 치료법으로서 고압산소요법의 영향을 모체와 태아의 위에서 평가할 필요가 있다. 본 실험에서는 다릅을 대한표를 실험동물로 사용하고 수매백서뿐 아니라 태자를 중심으로 태신귀결을 평가, 비교하고 성장성기행의 발생여부를 관찰하여 일산화탄소 중독에 의한 임신소양성의 총합적으로 평가하고자 하였다.

Sprague-Dawley계 백서를 수배시켜 기관형성의 초기단계에 있는 수배 제 11일에 1,800 ppm의 일산화탄소에 1시간 폭로시킨 군, 1,400 ppm의 일산화탄소에 2시간 폭로시킨 군, 그리고 1,400 ppm의 일산화탄소에 2시간 폭로시킨 후 15분에 45분간 3기압의 고압산소요법을 시행한 3개의 실험군을 대조군과 비교하였다.

수매백서를 위주로 하여 임신소양률, 수매기간 중 폐중증가율, 그리고 태자사암률을 지표로하여 임신귀결을 비교하였을 때는 1,400 ppm CO-2시간 폭로군에서만 일산화탄소중독에 의한 임신소양이 있는 것으로 판단되었다. 이에 비하여 태자의 성장발육을 지표로하여 비교하였을 때는 1,400 ppm CO-2시간 폭로군에서만 아니라 1,800 ppm CO-1시간 폭로군에서도 현저하게 태자성장의 저하가 관찰되었고 고압산소 치료군에서는 전반적인 신체성장은 정상범위내였으나 뇌의 산탄인성성장도를 사라주소의 성장이 관찰되었다.

체계적인 키형성併行법에 따라 적출된 태자를 관찰하였으나 두어하게 기형이라고 판단되는 태자는 발견되지 않았다.