Bull. Environ. Contam. Toxicol. 31, 85–92 (1983)

Effect of Cigarette Smoke and Carbon Monoxide Inhalation by Gravid Rats on the Conceptus Weight

Norihide Tachi and Mitsuko Aoyama

Department of Hygiene, Nagoya City University Medical School, Mizuho-ku, Nagoya 467, Japan

Cigarette smoking by pregnant women has been well known to exert deleterious effects on their infants. Fetal weight retardation has been especially recognized in almost all studies, whereas conflicting evidence exists for some of the other observed effects (SIMPSON 1957; LOWE 1959; FRAZIER et al. 1961; BUTLER et al. 1972). In laboratory animals also, cigarette smoke inhalation by the female during pregnancy has been reported to reduce the weight of fetuses (ESSENBERG et al. 1940).

Although the cause and mechanism of the fetal weight retardation induced by cigarette smoke have been proposed to be due to the direct effect of nicotine (BECKER et al. 1968), to fetal hypoxia produced by carbon monoxide (ASTRUP et al. 1972), and to poor weight gain by smoking women (DAVIS et al. 1976), the answer still remains unclear.

The present study was designed as a step towards explaining the phenomenon mentioned above. Fetal development at various stages was examined in pregnant rats which had inhaled either cigarette smoke or carbon monoxide at a concentration similar to cigarette smoke exposure. In addition, the most critical period of fetal weight decrease was determined.

MATERIALS AND METHODS

Animals: Primiparous Wistar rats were given light between 0600-1800h, and were maintained at 22.5-26.5°C. The day on which sperm were detected in the vaginal smear was designated as Day 0 of pregnancy. Water and food were available ad libitum, except for during the inhalation period. Animals were transferred to a metal mesh cage for exposure (1-2 rats/cage, 19 X 22 X 12 cm) around 0900h, and returned to their home cage (2-6 rats per cage, 45 X 25 X 20 cm) around 1700h.

Exposure: All the smoke passing through the cigarette filter from the complete combustion of 14 cigarettes ('Hi-lite', commercial brand of Japan Tobacco

Corporation), or 700 mL of carbon monoxide gas was introduced for 21 min into a chamber (95 X 80 X 70 cm) in which a maximum of 12 rats had been placed; they were kept there for another 60 min. Through this procedure, CO concentration in each chamber was maintained at 1000-1600 ppm. Animals in the control group were placed in the chamber, and further treatment such as pumping the room air was not administered. The rats were exposed twice daily (cigarette smoke and control inhalations; 1015-1136h, and 1500-1621h, and CO inhalation; 1036-1157h, and 1521-1642h).

Each rat in the group of cigarette smoke, CO, and control inhalation from Day 0 was sacrificed and autopsied after the morning exposure on Days 7, 14, and 21 of pregnancy. The uterus and ovary were removed, and the weights of the conceptus after removal of amniotic fluid and the number of fetuses and corpora lutea were recorded. On Day 7 of pregnancy, since it was difficult to remove conceptuses from the uterus, the uterine weight including embryo was determined. The weight per conceptus was calculated by dividing the litter weight by the litter size.

In another experiment, the exposure was done for only 7 of 21 days; from Days 0 to 6, Days 7-13, Days 14-20, and the variables described above were checked on Day 21 of pregnancy.

Duncan's new multiple range test was used for statistical analysis (STEEL & TORRIE 1980).

RESULTS

On Day 21 of pregnancy, the weight of the conceptus in mothers exposed to cigarette smoke (CIG) for 3 weeks was reduced to about 70% of that of the control group (CONT), as shown in Fig. 1. Similarly, the conceptus in CIG weighed significantly less than that of CONT on Day 14 of pregnancy, which confirmed the trend seen on Day 7, although not statistically significant, in the uterine weight including embryo. The fetal development was also retarded about 20% on Days 14 and 21 of pregnancy in animals exposed to carbon monoxide (CO), compared with CONT (Fig. 1). However, the uterine weight on Day 7 was not influenced by CO inhalation by the pregnant rat. When the conceptus weight in CIG was compared with that in CO, it was 10-15% higher in the CO group on Days 14 and 21 of pregnancy. The number of fetuses in CIG was smaller than CO only on Day 21 of pregnancy (Table 1). There was no difference in the number of corpora lutea (CL) at any stage except on Day 21 on which it was slightly lower in CONT than in

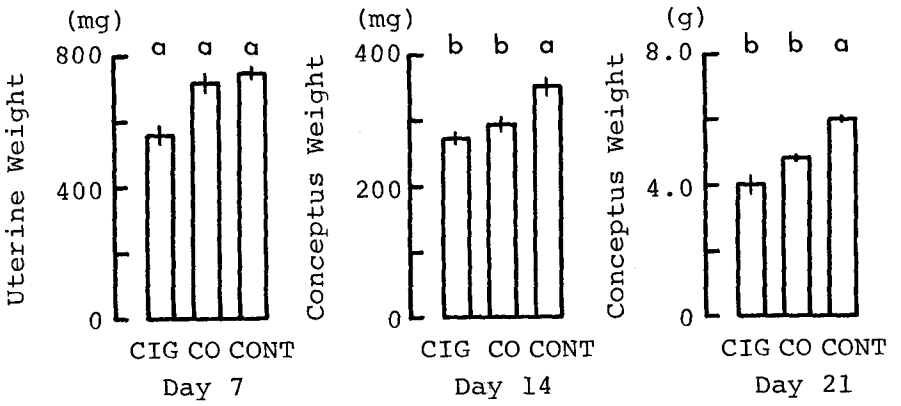

Fig. 1. Uterine weight on Day 7, and conceptus weight on Days 14 and 21 of pregnancy in animals exposed to cigarette smoke (CIG), carbon monoxide (CO), and room air (CONT). Each column represents mean ± S.E. Significant difference ($P<0.05$) between different letters on the column. N=8.

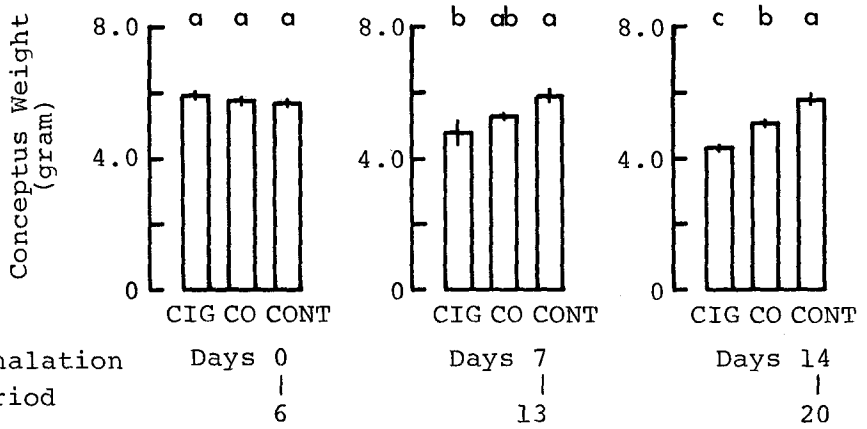

Fig. 2. Conceptus weight on Day 21 of pregnancy in mothers exposed to CIG, CO, and CONT from Days 0 to 6, from Days 7-13, and from Days 14-20. Number of samples is five except for the group of CONT inhalation between Days 7 and 13 in which the number is four.

Table 1. Increase in body weight of the pregnant rat, and number of fetuses and corpora lutea in the group of cigarette smoke (CIG), carbon monoxide (CO), and control (CONT) inhalation.

Day of pregnancy	Group	Increase in body weight (gram)	Number of fetuses	Number of corpora lutea
Day 7	CIG	- 8.8 ± 1.7^{c*}	13.4 ± 0.73^{a}	14.4 ± 0.56^{a}
	CO	10.4 ± 1.3^{b}	14.1 ± 0.47^{a}	15.0 ± 0.53^{a}
	CONT	17.0 ± 3.2^{a}	14.0 ± 0.37^{a}	14.3 ± 0.41^{a}
Day 14	CIG	19.4 ± 3.8^{b}	11.4 ± 0.75^{a}	15.3 ± 0.36^{a}
	CO	40.4 ± 3.4^{a}	11.4 ± 1.22^{a}	15.1 ± 0.61^{a}
	CONT	43.4 ± 3.1^{a}	13.5 ± 0.92^{a}	15.3 ± 0.59^{a}
Day 21	CIG	47.1 ± 8.5^{b}	10.1 ± 1.20^{b}	15.3 ± 0.36^{a}
	CO	93.8 ± 7.4^{a}	13.5 ± 0.75^{ab}	14.4 ± 0.46^{ab}
	CONT	107.8 ± 5.9^{a}	11.8 ± 1.08^{ab}	14.0 ± 0.26^{b}

Mean ± S.E.; * statistical significance between different letters (P 0.05). Number of animals is eight.

Table 2. Comparison of body weight gain by the pregnant rat from Day 0 to 21 of pregnancy, and number of fetuses and corpora lutea after 7-day inhalation exposure to CIG, CO, and CONT.

Duration of treatment	Treatment	Increase in body weight (gram)	Number of fetuses	Number of corpora lutea
Days 0-6	CIG	111.8 ± 5.9[a]	13.2 ± 0.37[a]	14.4 ± 0.24[a]
	CO	104.4 ± 5.7[a]	13.8 ± 0.37[a]	15.0 ± 0.63[a]
	CONT	113.8 ± 7.1[a]	13.0 ± 0.63[a]	14.4 ± 0.68[a]
Days 7-13	CIG	61.6 ±23.1[a]	13.4 ± 1.03[a]	15.2 ± 0.37[a]
	CO	105.8 ± 5.8[a]	12.5 ± 1.04[a]	14.3 ± 0.48[a]
	CONT	99.6 ± 3.0[a]	10.4 ± 1.12[a]	14.0 ± 0.84[a]
Days 14-20	CIG	67.8 ± 7.9[b]	13.8 ± 0.73[a]	14.4 ± 0.93[a]
	CO	110.6 ±11.9[a]	12.6 ± 0.68[a]	14.8 ± 0.37[a]
	CONT	102.8 ±12.0[a]	12.6 ± 2.01[a]	16.4 ± 1.12[a]

Number of animals is five except for the group of CONT exposed from Days 7-13, in which the number is four.

CIG. The weight gain in mothers, determined by the difference in the body weight between Day 0 of pregnancy and the day of autopsy, was always smallest in CIG. The weight gain in CO was a little less than in CONT, and there was a significant difference on Day 7.

The inhalation of CIG during the last 7 days of pregnancy most harmfully affected the fetal growth, while the exposure during the first 7 days failed to exert any effect (Fig. 2). The inhalation during the second 7 days of pregnancy resulted in intermediate reduction in the conceptus weight. A similar pattern was obtained by the 7-day exposure of CO (Fig. 2). The CIG inhalation during the second and last 7 days of pregnancy was however more hazardous than CO. No difference was observed in either number of fetuses or CL (Table 2). Maternal weight gain was significantly lower only in the group of CIG inhalation during the last 7 days of pregnancy, although non-significant reduction was shown in CIG during the mid-part of pregnancy.

DISCUSSION

Cigarette smoke inhalation by pregnant rats reduced conceptus weight on Day 21 of pregnancy in the present study, as documented by earlier authors (ESSENBERG et al. 1940; YOUNOSZAI et al. 1969; HAWORTH & FORD 1972). Moreover, the weight reduction effect was already evident by Day 14 of pregnancy. The inhalation during the last third of pregnancy was found to exert the most pronounced effect on fetal development, whereas that during the first third of pregnancy showed no significant effect. This observation is in accord with the results of REZNIK & MARQUARD (1980) and LOWE (1959) in rats and humans, respectively. Although the weight of the uterus, including embryo on Day 7 of pregnancy, was smaller in the cigarette group than those in the carbon monoxide and control groups, it is unknown at present which was affected by cigarette smoke; uterus, embryo, or both. However, since the exposure during the first third of pregnancy could not disturb the conceptus growth, it may be supposed that the reduction on Day 7 was due to the retarded uterine development.

ASTRUP et al. (1972) assumed that fetal hypoxia induced by carbon monoxide in cigarette smoke might be responsible for the retardation in fetuses. In the present study, carbon monoxide exposure also decreased the weight of conceptus on Days 14 and 21 of pregnancy, and the 7-days inhalation of the gas indicated a similar gradient in the fetal growth to cigarette smoke exposure. Therefore, the results of carbon monoxide

inhalation may imply that carbon monoxide is an important contributor to the effects of cigarette smoke. However, the finding that the effects of inhalation of carbon monoxide alone could not in any way match that of cigarette smoke in about a 10% difference, suggests that not only carbon monoxide can contribute to the fetal retardation, but that there may be other factor(s) such as nicotine (BECKER et al. 1968), cadmium (WEBSTER 1978), poor weight gain or appitite in mothers (YOUNOSZAI et al. 1969; DAVIS et al. 1976; PAPOZ et al. 1982).

The ratio of the number of surviving fetuses to corpora lutea, inversely reflecting the rate of abortion or infertilization, was lower on Day 21 of pregnancy in rats which had inhaled cigarette smoke for 3 weeks than others (67% vs. 93 and 83% for carbon monoxide and control groups, respectively). Albeit, further investigation should be undertaken to conclude the issue because of the broad variation among individual data.

In summary, both continuous inhalation by mothers of cigarette smoke and carbon monoxide lowered the weight of the conceptus on Days 14 and 21 of pregnancy, and the effect was more conspicuous in the group exposed to cigarette smoke than that to carbon monoxide alone. Therefore, the present study suggests that carbon monoxide is an important but not sole factor responsible for the adverse influence of cigarette smoke on the fetus. In addition, inhalation affected the fetus most deleteriously when performed during the last third of pregnancy, i.e. the phase of rapid fetal growth.

REFERENCES

ASTRUP, P., D. TROLLE, H.M. OLSEN, K. KJELDSEN: Lancet 2, 1220 (1972).

BECKER, R.F., C.D. LITTLE, J.E. KING: Am. J. Obstet. Gynecol. 100, 957 (1968).

BUTLER, N.R., H. GOLDSTEIN, E.M. ROSS: Brit. Med. J. 2, 127 (1972).

DAVIS, D.P., O.P. GRAY, P.C. ELLWOOD, M. ABERNETHY: Lancet 1, 385 (1976).

ESSENBERG, J.M., J.V. SCHWIND, A.R. PATRAS: J. Lab. Clin. Med. 25, 708 (1940).

FRAZIER, T.M., G.H. DAVIS, H. GOLDSTEIN, I.D. GOLDBERG: Am. J. Obstet. Gynecol. 81, 988 (1961).

HAWORTH, J.C., and J.D. FORD: Am. J. Obstet. Gynecol. 112, 653 (1972).

LOWE, C.R.: Brit. Med. J. 2, 673 (1959).

PAPOZ, L., E. ESCHWEGE, G. PEQUIGNOT, J. BARRAT, D. SCHWARTZ; Am. J. Obstet. Gynecol. 142, 870

(1982).
REZNIK, G., and G. MARQUARD: J. Environ. Pathol. Toxicol. 4, 141 (1980).
SIMPSON, W.J.: Am. J. Obstet. Gynecol. 73, 808 (1957).
STEEL, R.G.D., and J.H. TORRIE: Principles and procedures of statistics. A biometrical approach. 2 ed. Tokyo: McGrow-Hill Kogakusha, Ltd. (1980).
WEBSTER, W.S.: Arch. Environ. Health 33, 36 (1978).
YOUNOSZAI, M.K., J. PELOSO, J.C. HAWORTH: Am. J. Obstet. Gynecol. 104, 1207 (1969).

Accepted January 7, 1983