Effect of TCDD on Maternal Toxicity and Chorionic Gonadotropin

——Bioactivity in the Immediate Post-implantation Period of Macaque

GUO YU-MEI, WANG SHU-YI, WANG XIN-RU, AND LASLEY BILL*

Institute of Applied Toxicology, Nanjing Medical University, Nanjing 210029, China;

** Institute of Toxicology and Environmental Health, University of California at Davis, Davis, CA 95616*

The purpose of this experiment was to observe the alterations in bioactivity of chorionic gonadotropin (CG) associated with early fetal loss (EFL), induced by the environmental toxin TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) in the cynomolgus macaque. Ten of twelve females administered single doses of 1, 2 or 4 μg/kg TCDD on gestational day (GD) 12 had EFL from ten to twenty days later. Seven control animals treated only with the vehicle had normal pregnancies. Blood samples were repeatedly collected for hormone evaluation, from two days before treatment to thirty-one days following treatment. Immunoreactive monkey chorionic gonadotropin (mCG) was measured in serum using ELISA, and bioactive mCG was measured using a luminescence LH/CG bioassay. No change in immunoreactive mCG levels was detected as a result of TCDD, treatment, but bioactive mCG levels were significantly lower in TCDD-treated animals compared to controls. This change in bioactivity of mCG was also reflected in the ratio of mCG bioactivity to mCG immunoreactivity (B/I ratio) which began to rise in normal pregnancies by GD 20, but did not rise in TCDD treated animals. These results demonstrate that normal pregnancy in the monkey, as in humans, is characterized by a post-implantation change in the B/I ratio of CG. These findings therefore suggest that changes in the production of bioactive CG may be used as a biomarker of environmental toxicant exposures which lead to EFL.

INTRODUCTION

During very early pregnancy, the human trophoblast undergoes a series of developmental changes resulting in the production and secretion of the heterodimeric form of human chorionic gonadotropin (hCG), which stimulates steroidogenesis by the corpus luteum of the ovary through interaction with specific hormone receptors (Hearn, 1986). Recent studies have demonstrated that during the peri-implantation period of normal human pregnancy, the heterodimeric hCG, initially secreted, has limited capacity to transduce a biological signal through the LH/CG seceptor, but as implantation progresses, the hCG in circulation has increasing bioactivity. In contrast, the trophoblast of pregnancies which later end in spontaneous abortion tends to produce hCG with lower biological activity than the hCG of normal pregnancies, and this deficiency is most pronounced in pregnancies that abort early in the post-implantation period. These observations are consistent with the concept that the normal physiological changes which occur in trophoblast cells during implantation as well as abnormalities in this process are reflected in the biochemical properties of the trophoblastic hormones and that these changes in hormone production and secretion can be detected using assays of hormone bioactivity (Wang, Segal and Koide, 1988 and 1989).

Immunoreactive hCG has been used to detect early human pregnancies, and urinary

0895-3988/2000
CN 11-2914

hCG has been used to estimate the frequency of early fetal loss (EFL) in large populations of women in some epidemiologic studies (Wilcox *et al.*, 1988). The early human embryo is particularly sensitive to environmental hazards. One difficulty in associating EFL with environmental exposures is the relatively high spontaneous failure rate of pregnancy in non-exposed women (Wilcox *et al.*, 1988). Approximately one-third of all conceptions end in abortion with two-thirds of these being EFL which is detected only by the transient appearance of immunoreactive hCG. Only a pregnancy loss over the usual thirty percent spontaneous rate would be indicative of toxicant induced losses, since the pattern of hCG production in induced and spontaneous losses are not known to be different. A biomarker which identifies abnormal pregnancies immediately following exposure to an environmental hazard would improve the capability to detect environmentally-induced pregnancy losses.

In the present study, we utilized laboratory macaque to characterize the immunoreactivity and bioactivity of monkey chorionic gonadotropin (mCG) in normal pregnancies and in pregnancies which aborted following treatment with TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). TCDD is a very toxic chemical and is present at various levels, in the environment. A monkey model is required for such studies because of biological specificity in maternal-embryo signaling which is characteristic of closely related primate species. First, use tested the hypothesis that changes in the production of immunoreactive and bioactive CG in the peri-implantation period of normal pregnancy are similar in humans and in the macaque model. Second, use tested the hypothesis that the pattern of CG secretion, and in particular the pattern of secretion of bioactive CG are altered in pregnancies that fail because of a toxic exposure. The overall objective of the study was to demonstrate that the macaque is a useful model for investigating changes in the biochemical properties of CG which occur during early pregnancy and which may be altered as a result of an environmental exposure to toxicants.

MATERIALS AND METHODS

Animals

Female cynomolgus macaques (*Macaca fascicularis*) 6-12 years of age and weighing from 3.1-4.3 kg with a known reproductive history were purchased from the Primate Center University of California, Davis. All animals were housed and maintained according to the guidelines for laboratory animal care and were fed Purina Monkey Chow (25% protein) twice daily. Animal rooms, maintained on a 12∶12 hour light-dark cycle (lights on at 0600 hour), had a year-round temperature of approximately 22℃ and 60% relative humidity, and were equipped with an automatic watering system for provision of water ad libitum.

TCDD Treatments and Blood Sample Collections

Crystalline TCDD was purchased from Cambridge Isotope Laboratories, Inc (Andover, MA). (99.9% purity). TCDD was dissolved in acetone and diluted with corn oil to prepare doses of 1,2, and 4 μg/kg body weight ($n=4$ animals per dose group), and was administered as a single dose by nasogastric intubation. The control group ($n=7$) received the same dosing volume (1 mL/kg) of acetone-corn oil. Early morning urine samples were collected from cycle day 9 to cycle day 15 (cycle day 1 was the first day of vaginal bleeding) so as to identify the preovulatory estrone conjugates (E1C) peak and to determine the day of ovulation, defined as the day of the urinary E1C peak (Behboodi *et al.*, 1991). The day of the E1C peak was designated

gestational day (GD0). Animals were treated once with TCDD on GD 12, the initial day of pregnancy detection by ultrasonography.

Serum samples were collected from treated animals from treatment day −2 to day +16 (the day of TCDD treatment was treatment day 0), and for control animals from treatment day −6 to +16, then samples were collected every third day in all groups until treatment day 31.

Hormone Assays

Serum immunoreactive mCG was determined by ELISA (Munro *et al.*, 1997), serum bioactive mCG concentrations were determined by methods previously described (Guo *et al.*, 1999). The inter- and intra-assay errors expressed as coefficients of variation for the serum immunoreactive and bioactive mCG were 10.2%, 8.04% and 9.32%, 6.78% ($n=30$) respectively.

Statistics

Data are presented as $\bar{x} \pm s$. The comparison between the treatment groups and control group were analyzed by repeated-measures ANOVA. $P<0.05$ was considered to be significant.

RESULTS

As a result of single exposure to TCDD on GD 12, the pregnancy was subsequently aborted in 10 of 12 treated animals and EFL occurred from GD 22 to 32. The two surviving pregnancies occurred in the low dose group. All seven animals in the control group had normal term pregnancies (Table 1). Maternal toxicity was also observed following TCDD exposure (Table 2).

TABLE 1

Effect of TCDD on Early Fetal Loss (EFL) in Macaques

Dose (μg/kg)	Animal Number	EFL Number
1	4	2
2	4	4
4	4	4
control	7	0

Among the TCDD-treated animals, the immunoreactive mCG concentrations in the low-, mid- and high-dose groups did not differ from one another, and were not significantly lower than those in the controls ($P>0.05$) (Fig. 1A). Immunoreactive mCG in treated and control animals began to rise on the second day post treatment, and rose to peak values on treatment day 13 or 14, then declined steeply from peak values in treated and control animals and was undetectable in both the treated and control groups at about day 25 post treatment.

But bioactive mCG began to rise a day later in the treated animals than in control animals, and by day 8 post treatment bioactive mCG was three fold high in control animals than in treated animals (Fig. 1B). Bioactive mCG continued to be significantly lower in treated animals than in controls until treatment day 19 ($P<0.01$), when levels in both treated and control groups were near baseline (Fig. 1B). Al-

though there was variation between animals, the profiles of mCG bioactivity were similar in the different treatment groups.

TABLE 2

TCDD Maternal and Developmental Toxicity Following Exposure to TCDD

Dose (μg/kg)	Animal ID	Embryo Death[a]	Maternal Toxicity[a]								
			Weight Loss[b]	Poor Appetite	Alopecia	Anemia	Hepato-toxicity[c]	Facial & Ocular Changes[d]	Sloughed Fingernails	Dermatitis	Death
1	1	17	4% (33)	R		10			10		
	2	130 (S/B)	1% (26)		26	23	26				
	3	–	23% (12)	R			37				
	4	19	19% (27)	R			27	27			28
2	1	11	14% (50)	R	71		33	45		82	
	2	20	6% (44)	R							
	3	19	–	R							
	4	19	17% (53)	R			38	36			58
4	1	20	13% (37)			10		62			
	2	10	13% (34)	R		12	31				
	3	14	23% (17)	R	12	42		37	55		
	4	19	14% (32)			28	44	49	57	45	348

[a]Days post-TCDD treatment; S/B = stillbirth; R = reported.

[b]Maximum weight loss relative to pre-treatment weight (days post-treatment).

[c]Includes hepatomegaly and/or elevated liver enzymes.

[d]Includes swollen eyes, ocular discharge, inflammation, tearing, and/or loss of eyelashes.

When the ratio of mCG bioactivity to immunoreactivity (B/I ratio) was calculated for the control and treated groups, the B/I ratios of all groups were relatively low for the first six days following treatment (Fig. 1C). After that, the B/I ratio of control animals rose rapidly to reach a peak value on treatment day 10. The B/I ratio in treated animals remained significantly lower than those in control animals throughout the period of mCG production ($P < 0.001$) (Fig. 1C).

DISCUSSION

The data demonstrate that circulating concentrations of bioactive, but not immunoreactive CG are reduced in association with TCDD-induced EFL in the non-human primate model. These experimental findings are consistent with clinical observations that bioactive hCG is reduced in the immediate post-implantation period of human pregnancies that later abort spontaneously (Ho *et al.*, 1997b), and also are consistent with the results of experiments *in vitro* which demonstrate that normal growth and development of the human trophoblast is associated with a change in bioactivity of the hCG molecule (Ho *et al.*, 1997a).

The mechanism by which TCDD induces EFL in macaques is unclear. The primary endocrine alteration associated with embryo loss was lower levels of progesterone and estrogen, which could be a result of direct action of TCDD on the ovary or a reflection of the reduced secretion of bioactive mCG. The relatively long time from TCDD exposure to embryonic death (10 to 20 days) is consistent with a delayed effect on the conceptus that could involve the endocrinology of the maternal-fetal unit or be the result of a direct action on the embryo.

In the previous report describing changes in the B/I ratio of hCG during early

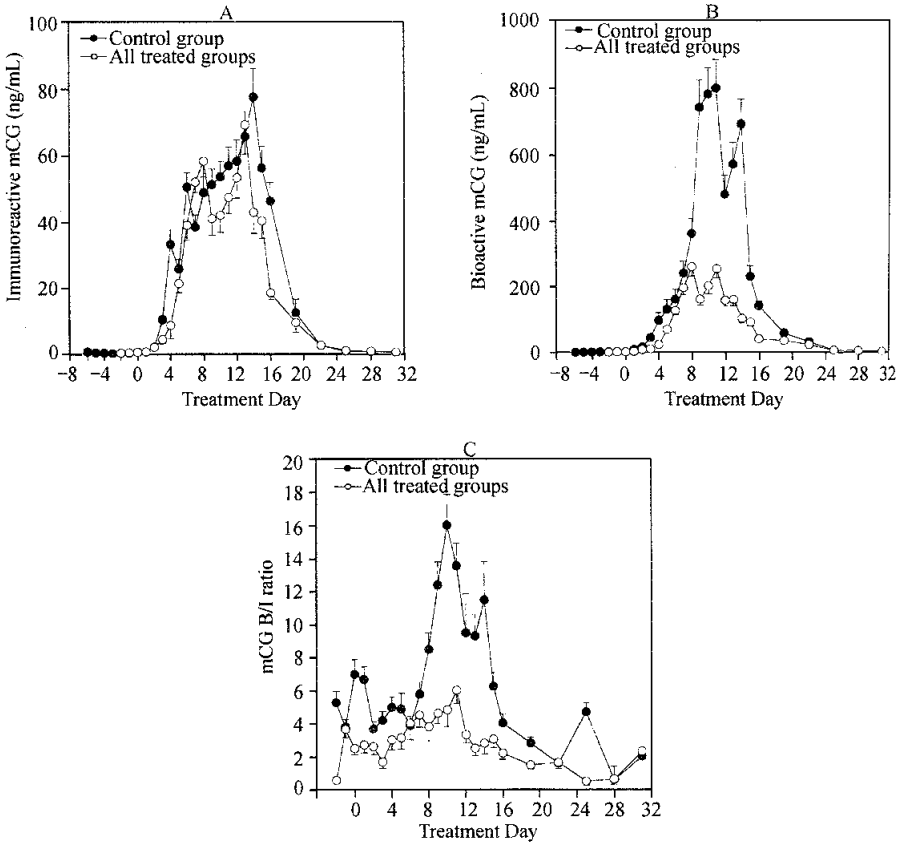

FIG. 1. $\bar{x} \pm s$ serum immunoreactive CG (A), bioactive CG (B), B/I ratio(C) profiles in the groups receiving 1 μg/kg, 2 μg/kg, 4 μg/kg TCDD relative to controls. Treatment day 0 corresponds to the day of TCDD treatment (GD 12).

human pregnancies, two well-characterized immunoassays for hCG were employed both of which are known to detect the intact hCG dimer (Ho *et al.*, 1997b and c). These methods and the use of the same standards in the immunoassays and bioassay enabled the authors to make direct comparisons between the amount of immunoreactive versus bioactive hCG in each sample. The immunoassay for mCG does not have a sensitivity or specificity which is comparable to the immunoassays that are available for hCG. For example, the immunoassays for mCG does not detect pregnancy until approximately day 12 post ovulation in macaques (Munro *et al.*, 1997), whereas hCG assays detect human pregnancy by day 9 post ovulation (Stewart *et al.*, 1993). By comparison to control values using the same assays it is apparent that TCDD did not suppress the immunoreactive forms of mCG at any dose, whereas the effect of TCDD on the bioactive forms of mCG was profound.

There are two reasons for expressing the values as a B/I ratio. The first is that there is a good deal of animal-to-animal (woman-to-woman) variation in CG production; a simple profile may not show a difference between normal and failing pregnancies. The second is that the authors wish to emphasize that while the amount of immunoreactive hCG is similar in normal and failing pregnancies, the difference is in the portion of the total hCG secreted that has full bioactivity. The primary objective of this study was to determine if the shift in B/I ratio associated with spontaneous preg-

nancy loss in human could be observed when EFL was induced in a non-human primate model as the result of an exposure to a toxicant. The results demonstrate that normal pregnancy in monkey, as in human, is characterized by a post-implantation change in the B/I ratio of CG. These findings suggest that the macaque is a useful animal model to conduct experiments on the physiologic and biochemical basis of this previously unrecognized aspect of embryo-maternal signaling. This monkey model can also be used for experiments in which exposures to toxicant at different stages of pregnancy demonstrate the utility of monitoring changes in the production of bioactive CG as a biomarker of environmental exposures which lead to EFL.

REFERENCES

Behboodi, E., Katz, D. F., Samuels, S. J., Tell, L., Hendrickx, A. G., and Lasley, B. L. (1991). The use of a urinary estrone conjugated assay for detection of optimal mating time in the cynomolgus macaque (*Macaca fascicularis*). *J. Med. Primatol.* **20**, 229.

Guo, Y. M., Hendrickx, A. G., Overstreet, J. W., Dieter, J., Stewart, D. R., Tarantal, A. F., Laughlin, L. A., and Lasley, B. L. (1999). Endocrine biomarkers of early fetal loss in Cynomolgus Macaques (*Macaca fascicularis*) following exposure to dioxin. *Biol. Reprod.* **60**, 707.

Hearn, J. P. (1986). The embryo-maternal dialogue during early pregnancy in primatess. *J. Reprod. Fertil.* **76**, 809.

Ho, H. H., Douglas, G. C., Qing, Q. F., Thirkill, T. L., Overstreet, J. W., and Lasley, B. L. (1997a). The relationship between trophoblast differentiation and the production of bioactive hCG. *J. Early Pregnancy: Biology and Medicine* **3**, 291.

Ho, H. H., O'connor, J. F., Tieu, J., Overstreet, J. W., and Lasley, B. L. (1997b). Characterization of hCG in normal and failing pregnancies. *J. Early Pregnancy: Biology and Medicine* **3**, 213.

Ho, H. H., O'connor, J. F., Overstreet, J. W., and Lasley, B. L. (1997c). Characterization of hCG peptide variants with a radio-receptor assay using recombinant human LH/CG receptors. *J. Early Pregnancy: Biology and Medicine* **3**, 204.

Munro, C. J., Laughlin, L. S., Illera, J. C., Dieter, J., Hendrickx, A. G., and Lasley, B. L. (1997). An ELISA for the measurement of serum and urinary chorionic gonadotropin concentrations in the laboratory macaque. *Am. J. Primatol.* **41**, 307.

Stewart, D. R., Nakajima, S. T., Overstreet, J. W., and Lasley, B. L. (1993). Enhanced ovarian steroid secretion before implantation in early human pregnancy. *J. Clin. Endocrinol. Metab.* **76**, 1470.

Wang, H., Segal, S. J., and Koide, S. S. (1988). Purification and characterization of an incompletely glycosylated form of human chorionic gonadortropin from human placenta. *Endocrinology* **123**, 795.

Wang, H., Segal, S. J., and Koide, S. S. (1989). Carbohydrate moieties of small placental hCG: requirement of mannose structure for biological activity. *Mol. Cell. Endocrinol.* **62**, 13.

Wilcox, A. J., Weinberg, C. R., O'connor, J. F., and Baird, D. D. (1988). Incidence of early loss of pregnancy. *N. Engl. J. Med.* **319**, 189.

(*Received September* 22, 1999 *Accepted December* 2, 1999)