Testes of rhesus monkeys exposed in utero and lactational period to 2,3,7,8-tetrachlorodibenzo-p-dioxin

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Introduction

Recently, the relationship between reduction of sperm number in human and environmental contamination of dioxin and dioxin related compounds has been investigated. The current tolerable daily intake (TDI) of dioxin and dioxin related compounds has been set at 4 pg TEQ/kg/day in Japan. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. Gray *et al.*[1]reported that a single oral dose of 200 ng/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats on day 15 of gestation resulted in abnormalities of reproductive organs in the female offspring. The maternal body burden at day of treatment was 97 ng/kg and it was 76 ng/kg at day 21 of gestation. Thus, the maternal body burden at LOAEL was estimated to be the mean of both values, approximately 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. This value was divided by an uncertainty factor of 10, and then the human TDI was established as described above. By TCDD exposure to pregnant rats at the LOAEL[2], as well as at lower level[3], reduction of sperm number in offspring has been reported. However, due to great differences in the biological half life of TCDD between human and rodents, the validity of LOAEL in human is questioned. To obtain more reliable LOAEL in the second generation, we initiated a long-term study in rhesus monkeys in 1999. Previously, we reported renal dysgenesis[4] and abnormal development of teeth[5, 6] in young of the TCDD exposed group. In this study, we examined effects of TCDD exposure in utero and lactational periods to development of the testes in young of rhesus monkeys.

Materials and Methods

Adult rhesus monkeys were mated, and females with confirmation of pregnancy by ultrasonography were given TCDD subcutaneously on day 20 of gestation at an initial dose level of 30 ng/kg (low dose group) or 300 ng/kg (high dose group). Controls received the vehicle. The lower dose level was set at about one third of the LOAEL body burden in rodents, and the higher one at about three times of the LOAEL. For maintenance of a certain body burden, 5% of the initial dose was given to dams every 30 days during pregnancy and lactation until 90 days after delivery. After weaning of the first newborn (F1a), the mothers were mated again, and TCDD was similarly treated but at an initial dose level of 20 ng/kg (low dose group) or 200 ng/kg (high dose group) on this time by consideration as TCDD accumulation in the maternal body. Maintenance treatment for second newborn (F1b) was same as F1a. In this study, totally ten postnatally died young (F1a and F1b aged 365 to 1297 days) and seven F1b sacrificed at the age about 850 days were autopsied. Testes were fixed in Bouin's fixative, and thin sections were routinely prepared for microscopic observations. In the cases where young died postnatally, the area of the seminiferous tubules was measured from the microphotograph.

Results and Discussion

In control testes of young that died postnatally, the seminiferous tubules and epithelium were well developed, but spermatogenesis was not recognized at the present age (Fig. 1a). In controls, the area of seminiferous tubules occupied about 58% of the testis tissue (Fig.2). On the other hand, the testes of young that died postnatally in the TCDD exposure group were severely edematous (Fig. 1b, c, d). The seminiferous tubules were compressed by the loosely expanded connective tissue. Thus, the area of the seminiferous tubules was significantly decreased compared with that of controls (Fig.2) The area of the seminiferous tubules of high dose group was about half of controls. In one case in the high dose group, a severely fibrous atrophic testis was recognized (Fig. 1d). In this case,

most part of the seminiferous tubules was replaced by the fibrous scar tissue. Diameter of the remaining seminiferous tubules varied greatly, and the lumen of some seminiferous tubules was obscure.

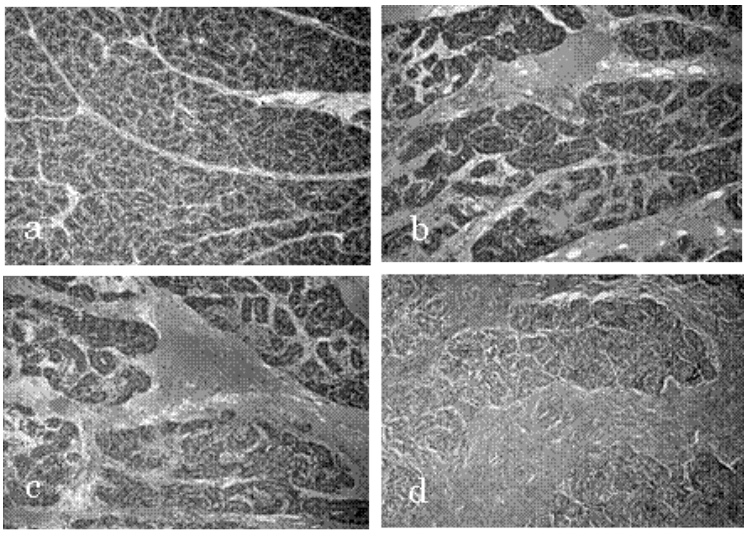

Fig.1
a: control, b: low dose group, c and d: high dose group

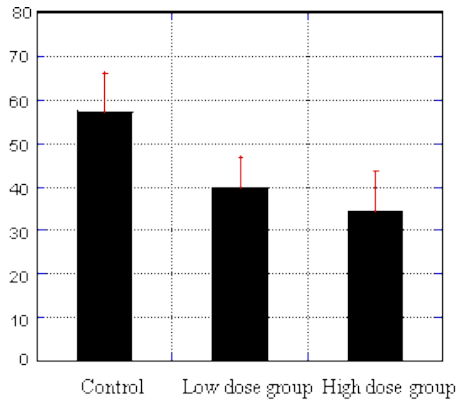

Fig.2 Area of the seminiferous tubules (%)

The histological findings of testes in sacrificed cases were similar with those of young that died postnatally (Fig.3). Some testes of the TCDD exposure group were edematous, like as shown in Fig.3. The level of edema varied among the testes, and it did not depend on dose level. In controls, the seminiferous epithelium was well developed and mature, and the spermatocytes were recognized, but spermatogenesis was not seen at the present age(Fig. 4). In TCDD exposure group, maturity level of the seminiferous epithelium was low, although level of maturity retardation did not depend on dose level.

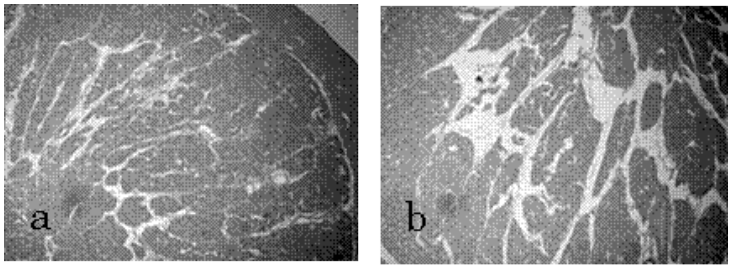

Fig.3 The testes in sacrificed group
a: control, b: low dose group

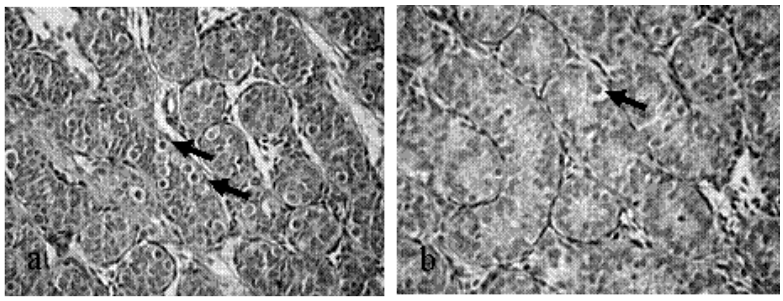

Fig. 4 High power micrograph of the testis tissue in sacrificed group.
a: control, b. low dose group, arrows: spermatocytes

It should not be concluded that these histological changes are absolutely due to effect of TCDD. However, developing testes of primates might be more sensitive to TCDD than those of rodents. The results of the present study suggest necessity to re-examine the validity of LOAEL in human.

Acknowledgements

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