Defects of the third molar teeth in rhesus monkeys prenatally and lactationally exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

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Introduction

Teeth are targets of developmental toxicity of dioxin. *In utero* and lactational TCDD exposure affects rat incisor and molar developmet[1,2]. In humans also tooth abnormalities were reported among populations exposed to dioxins[3]. We reported that pre- and postnatal exposure to TCDD affected development of deciduous and permanent teeth in rhesus monkeys[4]. The main abnormalities detected in stillborn and early postnatallyd died offspirng were missing deciduous incisors and molars. Observation upto approximatly 4 years of age revealed missing permanent incisors and premolars. In addition, cone-shaped or maldirected premolars were detected. This paper describes additional tooth findings in surviving 4.5-year-old offspirng .

Materials and Methods

Details of the materials and methods for this study are given elsewhere[4]. Briefly, adult female rhesus monkeys at the age of 5-7 years and weighing 4-6 kg were used. Female monkeys were mated with males for three days on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating periodwas disignated as day 0 of gestation (GD 0). On GD18 or 19, pregnancy was confirmed by an ultrasound device. Pregnant monkeys were divided into three groups, each consisting of approximately 20 animals. They were allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PND0). TCDD was dissolved in a mixture of toluene/DMSO (1:2, v/v) at a concentration of 300 ng/ml. Pregnant females were given TCDD subcutaneously into the back region on GD20 at an initial dose level of 30 or 300 ng/kg. The control animals received the vehicle in a volume of 1 ml/kg. For maintenance of a certain body burden, 5% of the initial dose, i.e. 0.6 or 6 ng/kg, was given to dams every 30 days during pregnancy andlactation until PND90.

Surviving offspring were examined at approximately 4.5 years of age for this study. They were anesthesized by intramuscular injection of ketamine at 10 mg/kg into the thigh before examination. Photographs were taken by an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo). Conventional intraoral radiographs were taken by a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto) with a charge coupled device (CCD) (Gendex Visualix, Dentsply International Inc., York, PA, USA).

Results and Discussion

In addition to the abnormal dental findings reported previously[4], the present observation revealed missing third molars in the 300 ng/kg. In controls all the permanent teeth including the third molars were radiographically recognizable at 4.5 years of age, although the third molars were not fully erupted. Figure 1 is a radiograph of the right molar portion of the lower jaw of the offspring No. 1 (PND1718) in the control group. The first and second molars have been fully erupted, and their crowns with cusps and roots are clearly visible. Although only a mesial half of the third molar (arrow) appears in this radiograph, its already calcified crown is clearly observable. In contrast, at least two (No. 31, PND1710 and No. 66, PND1618) of the eight surviving offspring in the 300 ng/kg group had missing third molars. In the offspring No. 31, the third molars on the left side in the upper jaw and on both sides in the lower jaw were not seen in radiographs which include enough areas distal to the second molars. Figure 2 is a radiograph of the right molar portion of the lower jaw of the offspring No. 31. In the presumptive position of the third molar (arrow), neither calcified crown nor root is observable. The radiograph of the left upper jaw did not reveal the position of the third molar. In the offspring No. 66, the presence of the upper left third molar could be confirmed, whereas the lower right one was apparently missing. There were two other cases (No. 39, PND1690 and No. 44, PND1695) with

possible missing third molars which could not be definitely confirmed. The size of the CCD unit, 23 x 38 mm, is fairly large when compared with the size of the oral cavity of a rhesus monkey at the age of 4.5 years. Therefore it was not easy to get clear radiographs of the distal portion of the upper and lower dental arches by placing the unit on the labial side of the molars.

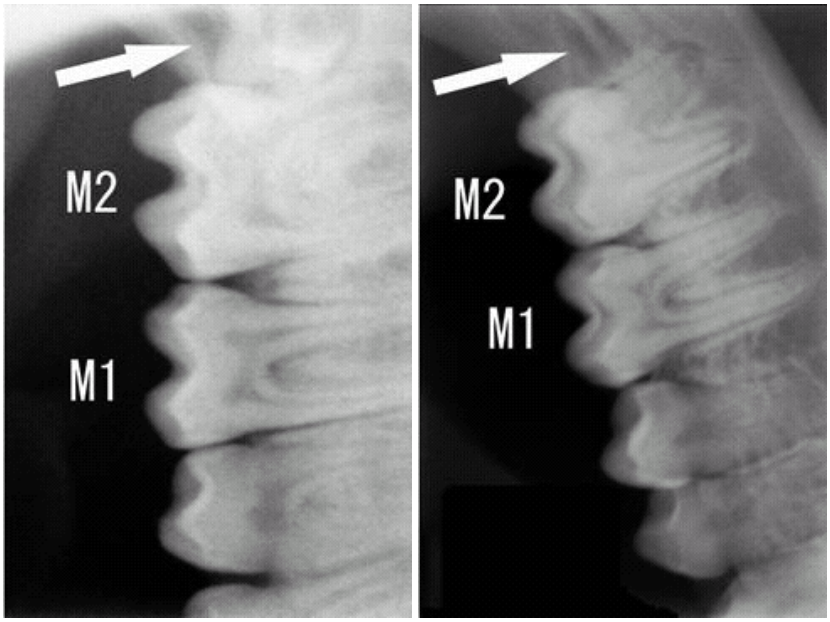

Figure 1.Lower right molars in No. 1 Figure 2. Lower right molars in No. 31

(Control group, PND 1718) (300 ng/kg group, PND 1710)

M1: the first molar; M2: the second molar; arrow: position of the third molar

The third molars in the rhesus monkey are still growing at the age of 4.5 years. Hence the final diagnosis of missing third molar should be done at the time of autopsy of these offspring.

In humans missing third molars are common among the general population. However, the third molars were invariably present in the parent monkeys used in the present study. Therefore missing third molars observed in the present study is considered to be caused by TCDD exposure.

Acknowledgements

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References

1 Kattainen H., Tuukkanen J., Simanainen U., Tuomisto J.T., Kovero O., Lukinmaa P.-L., Alaluusua S., Tuomisto J.and Viluksela M. (2001) Toxicol. Appl. Pharmacol. 174, 216.

2 Kiukkonen A., Viluksela M., Shalberg C., Alaluusua S., Tuomisto J. T., Tuomisto J. and Lukinmaa P.-L. (2002) Toxicol. Sci. 69, 482.

3 Alaluusua S., Calderara P., Gerthoux P.M., Lukinmaa P.-L., Kover, O., Needham L., Patterson D. G., Jr., Tuomisto J. and Mocarelli P. (2004) Environ. Health Perspect. 112, 1313.

4 Yasuda I., Yasuda M., Sumida H., Tsusaki H., Arima A., Ihara T., Tsuga K. and Akagawa Y. (2005) Reprod.

Toxicol. 20, 21.