Effects of Maternal Acetazolamide Treatment on Body Weights and Incisor Development of the Fetal Rat

Natsuki KOJIMA, Masato NAYA and Takashi MAKITA¹⁾

Toxicological Research Laboratories of Kyowa Hakko Kogyo Co., Ltd., Ube 755-8501 and ¹⁾Department of Veterinary Anatomy, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan

(Received 23 July 1998/Accepted 20 October 1998)

ABSTRACT. The incisor development of fetal rats on gestation day 19 was well correlated with their fetal weights. The number of odontoblasts in the mandibular incisors, an index of incisor development, increased more than that of the maxillary incisors with increase in fetal body weights. Maternal acetazolamide treatments were observed to suppress the mean fetal weight and to retard incisor development. A smaller incisor size, a thinner predentin layer, and fewer odontoblasts were characteristic of the acetazolamide group. There was also a good correlation between the fetal weights and the number of odontoblasts in the acetazolamide group. From these results, we postulated that the retarded incisor development of the fetal rats caused by the maternal acetazolamide treatment was related to their suppressed fetal weights. However, the regression coefficient of the fetal weights and the number of odontoblasts in the acetazolamide group was smaller than that of the vehicle control group. It may indicate that retarded incisor development in response to maternal acetazolamide treatment is to some extent independent of suppressed fetal weight.—**KEY WORDS:** acetazolamide, odontoblast, rat, retarded incisor development.

J. Vet. Med. Sci. 61(2): 143–147, 1999

The retarded incisor eruption of rat offspring is generally linked with the suppressed body weights of rat offspring [2, 3, 4, 6]. In our preliminary experiments, maternal treatment of high dose (800 mg/kg) of acetazolamide, a carbonic anhydrase inhibitor, was shown to suppress the growth of rat offspring and retard their incisor eruption. However, in our parallel study, we have observed that incisor eruption can be retarded without any suppression of the body weights of rat offspring when acetazolamide is orally administered at a dose of 200×2 mg/kg/day to pregnant rats (to be published). This discrepancy may be explained by the variable responses of the fetus, rather than the offspring, of the acetazolamide group. Therefore, the effects of this drug on fetuses are analyzed in this experiment.

MATERIALS AND METHODS

Test compound: Acetazolamide was purchased from Shigma Co., Ltd (St. Louis, Mo., USA.). A 0.3% sodium carboxy methyl cellulose solution was used for vehicle. Acetazolamide was suspended with the vehicle at a dosing volume of 1 ml/100 g body weight.

Animals and breeding: Slc:SD rats (SPF) were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were quarantined and acclimatized for 1 week after receipt and then used for this study at an age of 15 weeks. The animal room was maintained at a temperature of 22–26°C with a relative humidity of 45–65%, ventilated 20 times/hr, and lightened for 12 hr (7:00 a.m. to 7:00 p.m.). Animals were allowed free access to solid food and tap water. Copulation was regarded as established when a vaginal plug was found or sperm was present in the vaginal smears in the next morning after overnight mating. This day was considered

as day 0 of gestation. Female rats with confirmed copulation were stratified by body weight measured on day 0 of gestation and allocated randomly to each group.

Dosing: We administered acetazolamide orally to pregnant rats at a dose of 200 mg/kg twice a day from gestation day 7 to 11. In our parallel study, this dose was shown to retard incisor eruption with normal increases in body weight of offspring. As the control, 0.3% sodium carboxy methyl cellulose solution (1 ml/100 g body weight) was administered to pregnant rats in the same manner.

Test item: Fetuses removed from pregnant rats on days 15 and 19 of gestation were sacrificed under ether anesthesia and fixed in 10% buffered formalin for more than 1 week and weighed. The skull of male fetal rats on day 19 of gestation were decalcified in Plank-Rychlo solution, dehydrated, and embedded in paraffin. We used male fetuses for morphological examination because there were no sex differences in the retarded incisor eruption caused by the maternal acetazolamide treatment in the parallel study. Of 25 male fetuses from each group, about 100 consecutive sagittal sections of 5 μ m in thickness of incisors per fetus were stained by hematoxylin-eosin. The number of odontoblasts facing the predentin layer was counted as an index for incisor development of the fetuses. The number of odontoblasts of mandibular incisors was counted from the center of the incisal end to the apical end along the labial aspect. The mean number of odontoblasts was calculated from 10 consecutive sections for all the sections of each fetus, and the highest mean was regarded as representative data.

Statistical analysis: Body weight and number of odontoblasts were analyzed by the Student's *t* test [8]. The correlation coefficients and level of significance along with

Fig. 1. Incisors of fetal rats on gestation day 19 in the vehicle control group. Maxillary incisors (A) are elliptical and mandibular incisors (B) are longitudinal, with the latter showing more advanced developmental stage. The number of odontoblasts underlying the predentin layer were counted between the arrowheads. AB: ameloblasts, OB: odontoblasts, P: predentin layer, PR: protruding region with increase in fetal weights. $\times 88$. Insertion $\times 180$.

the linear regression were assessed according to Bancroft [1].

RESULTS

The incisor morphology of the male fetal rats on gestation day 19 of the vehicle control group was at the bell stage. Figure 1 shows the maxillary and mandibular incisors of fetal rats on that day. Ameloblasts and odontoblasts were well developed and predentin secreted by the odontoblasts was evident. Odontoblasts developed from the incisal end to the apical end along the labial aspect, but not along the lingual aspect. The maxillary and mandibular incisors were different in shape. The maxillary incisors were elliptical in form, and the mandibular incisors were longitudinal in form. With increase in the fetal weights, the predentin layers were thicker, and in the maxillary incisors the diameter was larger and the protrusion of incisal end was clearer. The mandibular incisors were further extended. Figure 2 shows that there was a correlation between the fetal weights and the number of odontoblasts both in the maxilla ($r^2=0.669$,

$P<0.01$) and mandibula ($r^2=0.712$, $P<0.01$). In the fetus of 1.5 g body weight, the number of odontoblasts in the maxillary and mandibular incisors was 76.4 and 108.7, respectively, and those in the fetus of 1.9 g body weight were 100.6 and 237.2. Those indicated that the number of odontoblasts in the mandibular incisors were 1.4 to 2.4 times larger than that of the maxillary incisors.

Fetal body weights in the acetazolamide group were lower than those of the vehicle control group on both gestation day 15 and 19 (Fig. 3). However, this suppression of fetal weight appeared to recover on day 19 (about 85% of control weight) of gestation as compared with that on day 15 (about 70% of control weight). A morphological examination of fetal incisors in the acetazolamide group revealed retarded incisor development (Fig. 4). Since fetal weights and incisor development in both the control and acetazolamide groups were variable, 3 samples of mean body weight were selected as representatives of each group. The size of the incisors was smaller, the predentin layer was thinner, and the number of odontoblasts was fewer than the vehicle control group (Fig. 5). The correlation between fetal weight and the number of odontoblasts both in the maxilla ($r^2=0.598$, $P<0.01$) and mandibula ($r^2=0.742$, $P<0.01$) is shown in Fig. 2. In the fetus of 1.5 g body weight, the number of odontoblasts in the maxillary and mandibular incisors was 75.4 and 107.8, respectively, and that in the fetus of 1.9 g body weight were 98.3 and 192.0. Those indicated that the number of odontoblasts in the mandibular incisors was 1.4 to 2.0 times larger than that of the maxillary incisors. The regression coefficient of the mandibula in the acetazolamide group was significantly smaller than that of the vehicle control group ($P<0.05$), but that of the maxilla was almost the same as that of the vehicle control group.

DISCUSSION

On gestation day 19, the size and shape of the incisor were remarkably different between the maxilla and mandibula. The maxillary incisors were elliptical in shape and much smaller than the mandibular incisors, which were longitudinal in shape. The number of odontoblasts, an index of incisor development, was well correlated with the fetal weights (Fig. 2). The regression coefficient in the mandibula was much larger than that in the maxilla. This difference in the regression coefficient between the maxilla and mandibula might be related to differences in the developmental stage of the fetal incisors on gestation day 19. As fetal weights increase, the length of the incisors increases and the number of odontoblasts also clearly increases in the mandibula, while the diameter of the incisors and the number of odontoblasts increase only slightly in the maxilla. Therefore, the number of odontoblasts in the mandibular incisors is a better index of incisor development than that in the maxillary incisors at this stage.

On the basis of these results, we examined the effects of maternal acetazolamide treatment on fetal weight and fetal incisor development. In the acetazolamide group, mean

Fig. 2. Correlation between the body weight and the number of odontoblasts in the fetal rats. The regression coefficients of the mandibular incisors are much higher than those of the maxillary incisors both in the vehicle control and acetazolamide groups. The difference was significant only in the mandibula when comparing the regression coefficients of the vehicle control and acetazolamide groups ($P < 0.05$). The arrows show the fetuses with the mean weights of each group. r^2 : Correlation coefficient.

Fig. 3. Body weights of fetal rats in control and acetazolamide groups. Fetal weights of rats in the acetazolamide group are significantly lower than those of the vehicle control group on both gestation day 15 and 19. The fetal weights tend to recover on gestation day 19 compared to those on day 15. Number in the columns and parentheses indicate the number of fetuses and that of litters, respectively. Bars indicate standard deviation. (: control group, ▨: acetazolamide group)

fetal weights were suppressed and the incisor development was retarded. The number of odontoblasts was smaller than that in the controls. Because there was a good correlation

between the fetal weights and the number of odontoblasts in the acetazolamide group, the retarded incisor development in the acetazolamide group appeared to be related to the

Fig. 4. Incisors of fetal rats on gestation day 19 with mean weights of the vehicle control group (1.81 g ; A,B) and the acetazolamide group (1.54 g ; C,D). In the acetazolamide group (C,D), the incisors are smaller and the predentin layer is thinner, and in the maxilla the protrusion is less clear. AB : ameloblasts, OB : odontoblasts, P : predentin layer, PR : protruding region $\times 52$.

Fig. 5. Number of odontoblasts of male fetal rats on gestation day 19 with mean fetal weights of each group (n=3). The number of odontoblasts in the acetazolamide group is reduced significantly (▨) compared to that in the vehicle control group (□). Bars indicate standard deviation.

lowered fetal weights. It has been reported that the body weights of mouse offspring correlate with their tooth size [5, 7], and that the retardation of incisor eruption in rat offspring is related to suppressed body weights [2–4, 6]. However we should take note of another factor; i.e. the suppressed fetal weights tended to recover on day 19 of

gestation compared to that on day 15 after withdrawal of acetazolamide on day 11. The same treatment of acetazolamide in pregnant rats does not suppress the body weights of offspring, although it does cause a retardation of incisor eruption. Together with the results of this experiment, we postulate that maternal acetazolamide treatments cause retarded incisor development accompanied by suppressed fetal weights, and that there is a recovery in the suppression of the fetal weight before delivery in response to the withdrawal of acetazolamide, while incisor development is retarded until their eruption. The lowered regression coefficient of the fetal weights and the number of odontoblasts in the acetazolamide group might be indicative of this lowered recovery of incisor development. The difference in recoverability between incisor development and fetal weight might indicate that maternal acetazolamide treatments cause a retardation of incisor development that is partially independent of the suppression of fetal weights.

REFERENCES

1. Bancroft, H. 1965. Introduction to Biostatistics. Harper and Row, New York.
2. Kato, I., Ushida, K., Kodama, R., Hosokawa, T., Huruhashi,

- T., Fukuda, S., Ono, H., Shiobara, Y., Takehara, A. and Takase, M. 1991. Reproduction studies of Sobuzoxane (II). Teratological study in rats. *Iyaku hin Kenkyu* 22: 644–655 (in Japanese).
3. Mizutani, M., Izutsu, M., Hashimoto, Y., Nagao, T., Matsuda, H. and Yoshida, R. 1986. Reproduction studies of dexamethason 17, 21-dipropionate (TSH-101) (I). Teratological study in rat. *Iyaku hin Kenkyu* 17: 310–326 (in Japanese).
 4. Mori, N., Sakanoue, M., Kamata, S., Takeuchi, M., Shimpo, K. and Tamagawa, M. 1982. Toxicological study of Buprenorphine (II). Teratogenicity in rats. *Iyaku hin Kenkyu* 13: 509–531 (in Japanese).
 5. Sasaki, Y., Nonaka, K. and Nakata, M. 1995. The strain effect of dam on intrauterine incisal growth in mouse fetuses. *J. Craniofac. Genet. Dev. Biol.* 15: 140–145.
 6. Sugiyama, O., Igarashi, S., Watabe, K., Watanabe, S. and Sato, T. 1992. Teratological Study of DWA2114R in rats. *Jpn. Pharmacol. Ther.* 20 (Suppl.): 159–175.
 7. Tenczar, P. and Bader, P.S. 1966. Maternal effect in dental traits of the house mouse. *Science* 152: 1398–1400.
 8. Winer, B. J. 1971. Statistical Principles in Experimental Design, 2nd. ed., McGraw-Hill, New York.