EFFECTS OF BARBITAL ON NEURO-ONCOGENESIS IN A TRANSPLACENTAL CARCINOGENICITY MODEL USING F344 RATS

Hiroyuki TANIGAWA, Hiroshi ONODERA* and Akihiko MAEKAWA**

*Division of Safety Assessment, Research Laboratories, Roussel Morishita Co., Ltd.,
1658 Ohshinohara, Yasu-cho, Yasu-gun, Shiga 520-23, Japan*

**Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences,
1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158, Japan*

***Department of Pathology, Sasaki Institute, 2-2 Kanda-Surugadai, Chiyoda-ku, Tokyo 101, Japan*

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ABSTRACT — Effects of barbitol (BB) on neuro-oncogenesis were examined in a rat transplacental carcinogenesis model. Pregnant F344 rats were divided into 7 groups. Dams in group I received subcutaneous injections of 10 mg/rat 1-butyl-1-nitrosourea (BNU) on the days 15, 18 and 21 of pregnancy and dams in groups II-IV, 1mg/rat BNU on the same time schedule. In addition to the treatment with BNU, dams in group IV were given 0.125% BB solution as their drinking water from the day 12 of pregnancy to parturition. Offspring in groups III and IV received 0.125% BB solution as drinking water from 4 weeks of age until the termination of the study. Animals in groups V and VI were given 0.25% and 0.125% BB solutions, respectively, in the peri- and postnatal period without BNU treatment. Dams in group VII received 250 mg/kg BB subcutaneously on the days 15, 18 and 21 of pregnancy. Offspring in all groups were observed until 105–116 weeks of age.

High yields of neurogenic tumors, such as gliomas and neurinomas, were observed in group I. In groups II, III and IV, single cases of a chordoma, a granular cell tumor, and a neurinoma and a malignant reticulosis, which are known to occur spontaneously, were noted, although no gliomas were found. No neurogenic tumors were observed in groups V–VII. With regard to lesions other than those in neurogenic organs, a significant increase in liver tumors was observed in group III compared to group II. In contrast, lung tumors were not found in group III, while they were observed in groups II and IV.

These results suggest that BB has no neuro-carcinogenic activity in the rat transplacental carcinogenesis model.

KEY WORDS : Barbitol, Transplacental or two-generation neuro-oncogenesis, Rat, N-Butyl-N-nitrosourea (BNU).

INTRODUCTION

It is generally accepted that environmental

chemicals, including pharmaceutical agents, agricultural chemicals, and food additives, are the most important causes of human cancers. Recently, the International Agency for Research on Cancer (IARC) evaluated the carcinogenic properties of almost 200 pharmaceutical chemicals,

Correspondence : Hiroyuki TANIGAWA at the above address.

and indicated that 20 were conclusively carcinogenic to humans and 52 were probably or possibly carcinogenic (Marselos and Vainio, 1991). The determination of carcinogenic risk to man has been judged on sufficient evidence derived from relevant and valid epidemiological studies, as well as evidence of carcinogenicity and other data from experimental animals.

Barbiturates have been widely prescribed as anticonvulsant, hypnotic, or sedative drugs. They are known to induce hepatic tumors in mice (Peraino *et al.*, 1973; Matsuyama and Amo, 1983) and also to promote the development of hepatic, renal and thyroid tumors after initiation with appropriate carcinogens in rats (Mori *et al.*, 1981; Diwan *et al.*, 1985; Hiasa *et al.*, 1982). Epidemiological studies, however, have revealed no clear evidence that barbiturates can cause the same tumors in humans (Clemmensen and Hjalgrim-Jensen, 1980; Shirts *et al.*, 1986; Olsen *et al.*, 1989), and the IARC has classified phenobarbital (PB), a long-acting barbiturate derivative, as "group 2B-possibly carcinogenic to humans", based on the available experimental and epidemiological data, in the IARC Monographs Programme (Marselos and Vainio, 1991).

The possibility that exposure to barbiturate medications, either in utero or during early childhood, may increase the risk of brain tumors occurring in children was suggested in a case-control study by Gold *et al.* (1978). This finding has raised some debate among epidemiologists (Annegers *et al.*, 1979; Gold *et al.*, 1979), and a potential risk has not been ruled out as yet. From experimental studies, PB was reported to have no promoting effect on neurogenic tumors induced by neonatal exposure to ethylnitrosourea (ENU) in mice and rats (Naito *et al.*, 1982; 1985) or those induced by transplacental exposure to methylnitrosourea (MNU) or ENU in rats (Diwan *et al.*, 1989; Walker and Swenberg, 1989). Until now, however, no report has been published with respect to promoting influence of other barbiturates on neuro-oncogenesis. In addition, there is no available information regarding the transplacental neuro-carcinogenicity of barbiturates in experimental animals.

Barbital (BB) has the basic chemical structure of barbiturates and is known to cause chromosomal aberrations in cultured Chinese hamster

cells (Ishidate and Odashima, 1977). Therefore, data on the influence of BB on neuro-oncogenesis in experimental animals are relevant to the risk assessment of this group of chemicals for man. The present study was undertaken to investigate any promoting potential of BB on neuro-oncogenesis in a transplacental carcinogenicity model using rats and also to examine the neuro-oncogenic effect by transplacental or two-generation exposure of rats.

MATERIALS AND METHODS

Animals and housing conditions: Male and female F344 rats (Charles River Japan Inc., Kanagawa) were mated overnight. Pregnancy was detected by the presence of a vaginal plug or sperm in vaginal smears, and the day of such findings was designated as the day 0 of gestation. Animals were maintained in an air-conditioned animal room at $21 \pm 1^\circ\text{C}$, $55 \pm 5\%$ relative humidity, and a 12-hour light/dark cycle with free access to basal diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo) and tap water.

Test compounds: 1-Butyl-1-nitrosourea (BNU) was purchased from Nakarai Tesque Inc. (Kyoto) and stored in a refrigerator (4°C) until use. Barbital, provided by Dr. M. Nakadate of National Institute of Health Sciences, was of reagent grade and its purity was 99.0%.

Experimental design: Figure 1 shows the experimental design. Pregnant rats were divided into 7 groups of 4 to 5 animals. Rats in group I were given subcutaneous injections of BNU (olive oil suspension) at a dose of 10 mg/rat on the days 15, 18 and 21 of gestation and animals in groups II-IV, 1 mg/rat on the same schedule. In addition to the BNU treatment, dams in group IV received 0.125% BB solution (dissolved in distilled water) as their drinking water from the day 12 of pregnancy to parturition. Offspring in groups III and IV were given 0.125% BB solution as drinking water from weaning (4-week-old) to the termination of this study. For groups V and VI, 0.25% and 0.125% BB solutions were given to rats as drinking water from the day 12 of pregnancy to parturition and also from weaning to the study termination without BNU treatment, respectively. In group VII, rats were given subcutaneous injections of 250 mg/kg BB on the days

Fig. 1. Experimental design for assessing neuro-oncogenic effects of BB in F344 rats.

↓, BNU (10 mg/rat, s.c.); ↓, BNU (1 mg/rat, s.c.); ⇓, BB (250 mg/kg, s.c.); □, 0.125% BB as the drinking water; ■, 0.25% BB as the drinking water

15, 18 and 21 of gestation. Three subcutaneous injections of 10 mg/rat BNU to pregnant rats is known to be sufficient for inducing high yields of neurogenic tumors in offspring (Maekawa and Odashima, 1975), and our preliminary study showed that subcutaneous injections of 1 mg/rat BNU was considered to be the lowest dose to influence effectively on neuro-oncogenesis. As to the dosage of BB, it was also confirmed that 0.25% BB solution was the maximum tolerated dose when it was given to rats as drinking water for 3 months, and 3 subcutaneous injections of 250 mg/kg BB was the maximum dose at which BB caused no abortion.

Histopathological examination: Dead or moribund animals (killed by ether euthanasia) during the course of study were autopsied completely and carefully examined for the presence of tumors. At 105–116 weeks of age, all surviving animals were autopsied after ether euthanasia. Tumors and all organs and/or tissues were fixed with buffered 10% formalin, and routinely prepared sections were stained with hematoxylin and eosin. Other special stains were used when indicated.

Statistical analysis: Intergroup differences in tumor incidences were statistically analyzed by Chi-square analysis or Fisher's exact probability

test.

RESULTS

Incidences of neuroectodermal tumors: Table 1 summarizes data for the incidence and type of neurogenic tumors in each group. In group I, tumors were detected from the early phase of the study and the final incidence was very high. The mean survival time of tumor-bearing animals was 52 weeks. In groups II–IV, tumors were found in only one or two animals in each group, and were without statistical significance with the survival times of tumor-bearing animals being 105–116 weeks. Furthermore, no neurogenic tumors were observed in groups V–VII.

Neurogenic tumors in group I were all macroscopically identifiable. Histologically, they were gliomas located in the cerebral hemispheres along with two neurinomas. The solitary neurogenic tumors observed in groups II and III were a chordoma and a granular cell tumor, respectively, and the two tumors found in group IV were a malignant reticulosis and a neurinoma. Histological types of gliomas in group I were astrocytomas, oligodendrogliomas and mixed gliomas. The astrocytomas were composed of medium-sized or large polygonal cells with abun-

Table 1. Incidences of neuroectodermal tumors and tumor latency in F344 rats given BNU and/or BB transplacentally and BB in the drinking water.

Group		I	II	III	IV	V	VI	VII
Treatment : dams	BNU(mg/rat)×3 ^{a)}	10	1	1	1	—	—	—
	BB(mg/kg)×3 ^{a)}	—	—	—	—	—	—	250
	BB(%) ^{b)}	—	—	—	0.125	0.25	0.125	—
	offspring BB(%) ^{c)}	—	—	0.125	0.125	0.25	0.125	—
No. of effective rats		20	14	25	29	34	19	31
(male/female)		(7/13)	(8/6)	(9/16)	(16/13)	(14/20)	(7/12)	(21/10)
No. of rats with neuroectodermal tumors (%)		14(70.0)	1(7.1)	1(4.0)	2(6.9)	0	0	0
(male/female)		(6/8)	(1/0)	(1/0)	(2/0)	—	—	—
Type of tumor : oligodendroglioma		9	0	0	0	0	0	0
mixed glioma		5	0	0	0	0	0	0
astrocytoma		2	0	0	0	0	0	0
malignant reticulosis		0	0	0	1	0	0	0
neurinoma		2	0	0	1	0	0	0
granular cell tumor		0	0	1	0	0	0	0
chordoma		0	1	0	0	0	0	0
Tumor latency (week)		53	116	105	114	—	—	—
(range)		(30-103)			(111-116)			

a) : Dams received 10 or 1 mg/rat BNU or 250 mg/kg BB subcutaneously on the days 15, 18 and 21 of pregnancy.

b) : Dams received 0.125 or 0.25% BB as the drinking water from the day 12 of pregnancy to parturition.

c) : Offspring received 0.125 or 0.25% BB as the drinking water from 4 weeks of age to the study termination.

dant eosinophilic cytoplasm, round nuclei and distinct nucleoli, and demonstrated indistinct cell margins. Oligodendrogliomas, composed of cells with compact round nuclei and a perinuclear halo in the cytoplasm, showed the so-called honeycomb patterns. Mixed gliomas were composed of mixtures of neoplastic astroglia and oligodendroglia elements consisting of 20–80% of each component. In some cases of astrocytomas or mixed gliomas, pseudo-palisading patterns of tumor cells around necrotic areas and numerous mitotic figures were observed, and the tumors were considered to be more anaplastic. Three peripheral nerve tumors found in this study contained areas resembling Antoni type A or B patterns and were diagnosed as malignant neurinomas, while the tumor detected in group IV was a microscopic lesion. The chordoma, granular cell tumors and malignant reticulosis were all histologically similar to spontaneous lesions (Maekawa *et al.*, 1990 ; Solleveld and Boorman,

1990).

Incidences of liver, lung and thyroid tumors :

Data for tumor incidences are summarized in Table 2. Liver tumors were detected in groups II, III, V and VII. The incidence in group III was slightly higher than that in group II and was significantly higher than that in group IV. Most of the tumors were hepatocellular adenomas, though one hepatocellular adenocarcinoma was observed in group III. Altered cell foci were found in all groups. Their incidences were increased in groups exposed to BB, and a significant increase was found in group IV in comparison to groups II and III. Lung tumors were detected in groups II, IV and VII, and the incidences in groups II and IV were significantly higher than that in group III. Histologically, diagnosed alveolar/bronchiolar carcinomas were detected in 2 animals of group II and group IV, but the other lesions were all adenomas. Thyroid tumors were observed in all groups except

Table 2. Incidences of liver, lung and thyroid tumors in F344 rats given BNU and/or BB transplacentally and BB in the drinking water.

Group		I	II	III	IV	V	VI	VII
Treatment : dams	BNU(mg/rat)×3 ^{a)}	10	1	1	1	—	—	—
	BB(mg/kg)×3 ^{a)}	—	—	—	—	—	—	250
	BB(%) ^{b)}	—	—	—	0.125	0.25	0.125	—
	offspring BB(%) ^{c)}	—	—	0.125	0.125	0.25	0.125	—
No. of effective rats		20	14	25	29	34	19	31
(male/female)		(7/13)	(8/6)	(9/16)	(16/13)	(14/20)	(7/12)	(21/10)
No. of rats with liver tumors (%)		0	1(7.1)	6(24.0)**	0	0(2.9)	0	1(3.2)
altered cell foci (%)		2(10.0)	1(7.1)	4(16.0)	12(41.4)*	10(29.4)	7(36.8)	7(22.6)
Type of tumor : adenoma		0	1	5	0	1	0	1
carcinoma		0	0	1	0	0	0	0
No. of rats with lung tumors (%)		0	3(21.4) [#]	0	6(20.7) [#]	0	0	1(3.2)
hyperplasia (%)		4(20.0)	1(7.1)	1(4.0)	0	0	1(5.3)	0
Type of tumor : adenoma		0	1	0	4	0	0	1
adenocarcinoma		0	2	0	2	0	0	0
No. of rats with thyroid tumors (%)		0	3(21.4)	3(12.0)	6(20.7)	3(8.8)	1(5.3)	3(9.7)
C-cell hyperplasia (%)		0	1(7.1)	1(4.0)	1(3.4)	1(2.9)	1(5.3)	0
Type of tumor : C-cell adenoma		0	3	3	5	3	1	3
C-cell carcinoma		0	0	0	1	0	0	0

a) : Dams received 10 or 1 mg/rat BNU or 250 mg/kg BB subcutaneously on the days 15, 18 and 21 of pregnancy.

b) : Dams received 0.125 or 0.25% BB as the drinking water from the day 12 of pregnancy to parturition.

c) : Offspring received 0.125 or 0.25% BB as the drinking water from 4 weeks of age to the study termination.

* : Significantly different from groups II and III at $P < 0.05$.

: Significantly different from group III at $P < 0.05$.

** : Significantly different from group IV at $P < 0.01$.

group I, although the incidences did not significantly differ among groups. Histologically, all tumors were C-cell tumors and no follicular cell neoplastic lesions were found.

Incidences of tumors in other organs and/or tissues: Table 3 shows data for incidences of tumors other than neuroectodermal, liver, lung and thyroid lesions. Endocrine tumors, leukemias, mammary tumors, testicular tumors, uterine tumors, preputial/clitoral gland tumors, or mesotheliomas, were found in all groups, but without any intergroup differences. Histologically, they were all similar to reported spontaneous lesions, known to occur commonly in this strain of rats (Maekawa, 1990; Maekawa *et al.*, 1983; Tanigawa *et al.*, 1987).

DISCUSSION

Gold *et al.* (1978) reported an increased risk of brain tumors in children exposed to barbiturates in a case-control study. When they combined prenatal and childhood exposures to barbiturates, they obtained an odds ratio of 3.0 when using healthy children as controls and a significant odds ratio of 5.5 when using other cancer patients as controls. From these results, they suggested that prenatal or childhood exposure to barbiturates may play a role in the development of as many as 8% of the brain tumors in children. Annegers *et al.* (1979), however, reported a negative association of prenatal or childhood barbiturate exposure with childhood brain tumors in their cohort studies. Follow-up studies of

Table 3. Incidence of other tumors in F344 rats given BNU and/or BB transplacentally and BB in the drinking water.

Group		I	II	III	IV	V	VI	VII
Treatment : dams	BNU(mg/rat)×3 ^{a)}	10	1	1	1	—	—	—
	BB(mg/kg)×3 ^{a)}	—	—	—	—	—	—	250
	BB(%) ^{b)}	—	—	—	0.125	0.25	0.125	—
offspring	BB(%) ^{c)}	—	—	0.125	0.125	0.25	0.125	—
No. of effective rats		20	14	25	29	34	19	31
(male/female)		(7/13)	(8/6)	(9/16)	(16/13)	(14/20)	(7/12)	(21/10)
No. of rats with tumors (%) in								
pituitary gland : adenoma		1(5.0)	4(28.6)	3(12.0)	3(10.3)	1(2.9)	3(15.8)	3(9.7)
adrenal gland : pheochromocytoma		2(10.0)	1(7.1)	0	4(13.8)	0	0	1(3.2)
malignant								
pheochromocytoma		0	0	0	0	0	0	1(3.2)
pancreas : insuloma		0	0	1(4.0)	1(3.4)	0	0	1(3.2)
hematopoietic organ : leukemia		0	4(28.6)	3(12.0)	4(13.8)	5(14.7)	0	3(9.7)
thymoma		0	0	0	0	0	0	1(3.2)
spleen : hemangioendothelioma		0	0	0	0	0	0	1(3.2)
duodenum : fibrosarcoma		0	0	0	0	1(2.9)	0	0
colon : adenoma		0	0	0	0	0	0	2(6.5)
testis : interstitial cell tumor		1(14.3)	7(87.5)	7(77.8)	15(93.8)	14(100)	6(85.7)	20(95.2)
ovary : granulosa cell tumor		0	0	0	1(7.7)	0	0	0
uterus : endometrial stromal polyp		0	1(12.5)	1(6.3)	2(15.4)	4(20.0)	6(50.0)	2(20.0)
endometrial hyperplasia		0	1(12.5)	0	1(7.7)	0	0	0
adenoma		0	0	0	0	0	1(8.3)	0
adenocarcinoma		1(15.4)	0	2(12.5)	0	0	0	0
vagina : stromal sarcoma		0	0	2(12.5)	0	0	0	0
mammary gland : fibroma		0	3(21.4)	0	1(3.4)	1(2.9)	0	0
fibroadenoma		0	2(14.3)	0	1(3.4)	2(5.9)	1(5.3)	0
adenoma		0	1(7.1)	0	1(3.4)	0	0	1(3.2)
adenocarcinoma		0	0	0	0	0	0	1(3.2)
skin : papilloma		0	2(14.3)	1(4.0)	0	0	0	0
sebaceous adenoma		0	0	0	1(3.4)	0	0	1(3.2)
subcutis : fibroma		0	0	2(8.0)	0	2(5.9)	0	0
lipoma		0	0	1(4.0)	0	0	0	0
histiocytic sarcoma		0	1(7.1)	0	0	0	0	0
preputial/clitoral gland :								
adenoma		0	1(7.1)	0	3(10.3)	2(5.9)	0	3(9.7)
squamous cell carcinoma		0	0	1(4.0)	0	0	0	1(3.2)
peritoneum : mesothelioma		0	0	1(4.0)	1(3.4)	1(2.9)	0	0

a) : Dams received 10 or 1 mg/rat BNU or 250 mg/kg BB subcutaneously on the days 15, 18 and 21 of pregnancy.

b) : Dams received 0.125 or 0.25% BB as the drinking water from the day 12 of pregnancy to parturition.

c) : Offspring received 0.125 or 0.25% BB as the drinking water from 4 weeks of age to the study termination.

patients treated with barbiturates for epilepsy showed a higher level of brain tumors than expected (White *et al.*, 1979; Clemmensen and Hjalgrim-Jensen, 1980; Shirts *et al.*, 1986; Olsen *et al.*, 1989), but in each of these cases, most brain tumors were detected at an early period after the diagnosis of initial epilepsy seizures, and the risk was noted to decline as the duration of barbiturate-use increased. The authors therefore suggested that the association was more likely due to inclusion of patients with undiagnosed slow-growing brain tumors that led to the seizures, rather than to an etiological role for barbiturates. On the other hand, Shirts *et al.* (1986) noted in their cohort study of epilepsy patients that the incidence of brain tumors diagnosed 5 or more years after onset of seizures was also higher than expected. Goldhaber *et al.* (1990) further concluded that their case-control study could not entirely rule out the possibility that prolonged exposure to barbiturates might either promote the growth of preexistent tumors or initiate tumors *de novo*. As mentioned above, the possibility that exposure to barbiturates is able to increase the risk for childhood brain tumors has not been excluded epidemiologically.

In experimental animals, it is well known that nitrosoureas can induce neurogenic tumors in rats, especially when administered during fetal or neonatal period at which the developing nervous system is highly susceptible to neuro-oncogenic compounds (Rice, 1981; Maekawa and Mitsumori, 1990). The several experiments have been carried out to elucidate the promoting potential of PB for neuro-oncogenesis in rats and mice after transplacental or neonatal administration of nitrosourea compounds (Diwan *et al.*, 1989; Walker and Swenberg, 1989; Naito *et al.*, 1985). Diwan *et al.* (1989) reported that postnatal administration of PB had no effect on the development of neurogenic tumors in F344 rats initiated transplacentally by MNU, though thyroid follicular cell neoplasms and preneoplastic hepatocellular lesions were increased. Walker and Swenberg (1989) also demonstrated a lack of promoting activity of PB on neuro-oncogenesis in male F344 rats transplacentally exposed to ENU. In addition, Naito *et al.* (1985) described that postnatal PB administration inhibited the develop-

ment of gliomas in WF rats treated neonatally with ENU. From these results, it can be concluded that PB does not enhance the development of neurogenic tumors in rat two-stage neuro-carcinogenicity models. However, no reports have been available for BB, a long-acting barbiturate like PB, on promoting activity for neuro-oncogenesis in rodents.

In the present study, a high incidence of neurogenic tumors was detected in rats receiving 10 mg/rat of BNU transplacentally (Group I), and the incidence and histological types were similar to those described previously by Maekawa and Odashima (1975). In groups II-IV, however, no gliomas were found in animals receiving 1 mg/rat BNU transplacentally. In the present study, we selected 3 subcutaneous injections of 1 mg/rat BNU as an initiator, which was considered to be the lowest dose effective for neuro-oncogenesis from our preliminary study. The result, however, shows that 1 mg/rat BNU given transplacentally was insufficient for complete neuro-carcinogenicity in the present experimental condition. Although no gliomas were detected in groups II-IV, the other tumors, such as lung adenocarcinomas and hepatic tumors, in group II were presumably BNU-treatment associated since their spontaneous occurrence is rare in this strain of rats (Haseman *et al.*, 1984; Maekawa, 1990; Maekawa *et al.*, 1983), and they are known to be inducible by N-alkyl-nitrosoureas such as MNU and ENU, analogues of BNU (Maekawa *et al.*, 1984; Tsuda *et al.*, 1983). These results could not completely exclude the possibility that transplacental administration of 1 mg/rat BNU acted as an initiator for the nervous tissues while no gliomas were induced and BB exerted no promoting effect on the present transplacental neuro-oncogenesis model. Further studies using a higher dose of BNU or other potent neuro-carcinogens, such as ENU, however, may be necessary to lead the more reliable conclusion. The other tumors, such as a chordoma, granular cell tumor, and malignant reticulosis, which were found, are all known to spontaneous neurogenic tumors in this strain of rats, albeit at very low incidences (Maekawa *et al.*, 1984; Solleveld and Boorman, 1990), and there is no indication that these tumors are induced by neuro-oncogenic chemicals (Maekawa and Mitsumori, 1990). The

possibility that the neurinoma in group IV was caused by BNU and/or BB can not be completely ruled out, since this tumor is known to be induced by BNU (Maekawa and Odashima, 1975) and 2 tumors are also detected in group I of the present study. However, the long latency period and the tumor size point to a spontaneous origin.

Until now, there has been no report, to our knowledge, regarding the effects of barbiturates on neuro-oncogenesis when exposure is transplacental or from early postnatal period in rats, although a negative association of BB or PB exposure with development of neurogenic tumors in long-term carcinogenicity studies in mice and rats has been repeatedly documented (Matsuyama and Amo, 1983; Imai *et al.*, 1980; Peraino *et al.*, 1973; Ponomarev and Tomatis, 1976; Rossi *et al.*, 1977). From the results of transplacental carcinogenicity studies in rats, Swenberg *et al.* (1972) suggested that neurogenic tumors of adult humans, as well as of children, may be due to transplacental exposure to neuro-oncogenic agents. Rice and Ward (1982) also mentioned that women of child-bearing age and children may be at higher risk for tumors of nervous system. Therefore, transplacental administration of chemicals, suspected of being neuro-oncogenic, has particular advantages for experimental testing for potential carcinogenicity to man. In the present study, we therefore administered the maximum tolerated dose of BB in the drinking water or the maximum dose of BB which caused no abortion to rats transplacentally and/or postnatally. Studies on placental transfer have indicated that most drugs administered during pregnancy cross the placenta and enter the circulation of fetus and equilibrium between fetal and maternal circulation is achieved rapidly (Mirkin, 1973). The same results have been reported on barbiturates, such as BB, PB and pentobarbital, in mice or rats (Karasawa *et al.*, 1967; Kuwajima *et al.*, 1969; Waddell, 1972; DeVane *et al.*, 1991). It is no doubt that the fetuses in this study were exposed to BB, although we did not know the exact level of fetal exposure to BB. In the present study, no neurogenic tumors were detected in groups V–VII. It has been well known that spontaneous occurrence of neurogenic tumors is very rare in F344 rats and the incidence of spontaneous gliomas is reported

to be 0.4–1.7% in males and 0.5–0.7% in females (Maekawa *et al.*, 1983; Haseman *et al.*, 1984). From this result, we conclude that BB has no neuro-carcinogenic activity by transplacental or two-generation exposure of rats, though a non-treated control group was not provided in the present study.

In the present study, liver tumors were increased in group III and decreased in group IV. It is known that BB promotes rat hepatocarcinogenesis by N-2-fluorenyl-acetamide (Mori *et al.*, 1981) and N-nitrosodiethylamine (Diwan *et al.*, 1985), although it does not enhance the occurrence of spontaneous liver tumors in rats (Imai *et al.*, 1980). The result for group III indicates that postnatal BB administration also acts as a promoter for liver cells initiated by transplacental BNU exposure. The explanation that the reduction of carcinogenic process in rats, when barbiturates and hepatocarcinogen are simultaneously administered, would be attributable to stimulation of hepatic drug-metabolizing enzymes (Ishidate *et al.*, 1967; Peraino *et al.*, 1971; Mushlin and Peraino, 1974; Weisburger *et al.*, 1975; Narita *et al.*, 1980; Diwan *et al.*, 1985), would be also applicable to our findings of group IV. Interpretation of the data for lung tumors, with significant induction in groups II and IV, but not group III, is not as simple. It is reported that BB and PB have no enhancing effects on the spontaneous occurrence of lung tumors in mice and rats (Matsuyama and Amo, 1983; Peraino *et al.*, 1973; Ponomarev and Tomatis, 1976; Rossi *et al.*, 1977; Imai *et al.*, 1980) and PB does not promote lung oncogenesis in rats initiated by nitrosodiethylamine (Diwan *et al.*, 1985; Maekawa *et al.*, 1992). Further studies are necessary to clarify how BB might exert enhancing or inhibiting effects on lung tumorigenesis. In the present study, no follicular tumors of the thyroid were found in any group. BB would thus appear to lack complete carcinogenic potential for this tissue, while it is well known that barbiturates promote development of thyroid tumors in rats (Diwan *et al.*, 1985; Hiasa *et al.*, 1982).

In conclusion, the present investigation did not provide any evidence of a carcinogenic activity of BB for rat neuro-oncogenesis by transplacental or two-generation exposure. Based on our and other investigator's results indicating the

negative association of BB- or PB-exposure with neurogenic tumors in experimental animals, barbiturates may not have neuro-oncogenic potential in man.

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