Mutation Research, 295 (1993) 113–123

MUTAGI 09096

Life span reduction and carcinogenesis in the progeny of rats exposed neonatally to 5-bromo-2′-deoxyuridine

Vladimir N. Anisimov and Galina Yu. Osipova

Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, St. Petersburg, Russia

(Received 5 November 1992)
(Revision received 9 February 1993)
(Accepted 10 May 1993)

Keywords: 5-Bromo-2′-deoxyuridine; Ageing; Carcinogenesis; Rat offspring

Summary

Outbred LIO rats were given subcutaneous injections (3.2 mg) of a synthetic analogue of thymidine, 5-bromo-2′-deoxyuridine (BrdUrd) on days 1, 3, 7 and 21 postnatally. At 3 months, the treated males and females were mated to generate F_1 progeny. The mean life span decreased by 31.6% and 9.1% in male rats and by 21.1% and 7.2% in female rats exposed to BrdUrd and in their offspring, respectively. Exposure to BrdUrd increased the aging rate of the rats and of their progeny. Age-related changes in the length of the estruc cycle and in the incidence of persistent estrus and/or anestrus were observed earlier in female rats exposed neonatally to BrdUrd and in their offspring compared to controls; also, developmental stigmas were observed in the offspring of rats exposed neonatally. The incidence of total and malignant tumors was increased in rats that had received BrdUrd as well as in their progeny. Our observations on the decrease in mean and maximum life span, the increase in aging rate, the acceleration of age-related changes in female reproductive system function, and the increase in tumor incidence and decrease in tumor latency in rats exposed to BrdUrd in early life suggest that this system could serve as a model of accelerated aging. These effects persist at least to the next generation.

5-Bromo-2′-deoxyuridine (BrdUrd), a thymidine analogue, can substitute for thymidine in DNA and induce many biological effects both in vitro and in vivo. Thus, it has been reported both to enhance (Stockdale et al., 1981) and to inhibit (Tapscott et al., 1989) cell differentiation, inhibit DNA synthesis and cell replication in vivo (Weghorst et al., 1991) and in vitro (Morris et al., 1989). BrdUrd induces viruses in cell culture (Lieber and Todaro, 1973) and teratogenic effects in mice and hamsters (DiPaolo, 1964; Ruffolo and Ferm, 1965). Since it is incorporated exclusively into cellular DNA in the place of thymidine, BrdUrd is widely used for measuring the rate of DNA synthesis (Lewis and Swenberg, 1982) and sister-chromatid exchange (Morris, 1991).

BrdUrd has been shown to be mutagenic in cellular systems (Morris, 1991), to produce sec-

Correspondence: Dr. V.N. Anisimov, Chief, Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, 68 Leningradskaya Str., Pesochny-2, St. Petersburg 189646, Russia.
Tel.: (812) 237-86-07; Fax: (812) 237-89-47.

ond generation mutants in trhe eukaryotic alga *Volvox cartesi* (Kirk et al., 1987), and to have a miscoding effect in cell-free systems (Trautner et al., 1962). Being incorporated into replicating DNA in place of thymidine, BrdUrd persists over a long time in several tissues (Likhachev et al., 1983; Ward et al., 1991). Assuming that there is no 5-bromouracil repair in rat DNA (Lindahl, 1982), and as BrdUrd after incorporation pairs with guanine when present as the enol tautomer, base pair substitution mutations are expected to occur (GC → AT and AT → GC transitions) during subsequent DNA replication (Davidson et al., 1988). A comprehensive review of the genetic toxicology of BrdUrd in mammalian cells has been published recently (Morris, 1991).

In our previous experiments it was shown that neonatal exposure of rats to BrdUrd was followed by slight carcinogenic effects, by an increased incidence of chromosome aberrations, by an acceleration of age-related disturbances in reproductive system function and aging rate and by a reduction of life span of the animals (Napalkov et al., 1989a; Anisimov and Osipova, 1992). Taking into consideration these results as well as the data on teratogenic and mutagenic potential of BrdUrd it is important to evaluate the effect of exposure of animals to this nucleoside analogue on life span, reproductive function and tumor development in their progeny.

It was shown that prenatal exposure of rodents to chemical carcinogens may result in an increased risk of tumor development in subsequent generations (Napalkov et al., 1989b). Further suggestive evidence for the role of prezygotic events in increasing the risk of cancer in otherwise untreated successive generations came from experiments where male rats were treated with ethylnitrosourea before mating with untreated females (Tomatis et al., 1981; Turusov et al., 1988). Exposure of male rodents to X-irradiation before mating also increased the risk of cancer development in offspring (Nomura, 1991; Anisimov et al., 1992). Recent observations by Gardner et al. (1990) of an increase in leukemia incidence in children whose fathers had worked on a nuclear plant before conception of their children has attracted renewed attention to the possibility of germ-line transmission of carcinogenic effects of exogenous agents. Intravenous infusions of BrdUrd are used in studies of proliferative activity in human cancers (Christov et al., 1991) and it also was used as radiosensitizer in the therapy of cancer patients (Szybalsky, 1974). However, it remains unclear whether the initiating effect of BrdUrd increases the susceptibility of progeny to carcinogenesis.

In this paper the results of a study on the effect of neonatal exposure of rats to BrdUrd on survival parameters, tumor development and the functional status of the reproductive system in their F_1 progeny are presented.

Materials and methods

Chemical

BrdUrd, from Sigma Chemical Co. (USA), 100% pure, was stored at +4°C.

Animals

Outbred LIO male and female rats from the Animal Department of the N.N. Petrov Research Institute were used. The mean life span of both males and females of this strain is about 23 months, and the maximum life span 35 months; the spontaneous tumor incidence is 27% in males and 47% in females (Anisimov et al., 1989).

After mating and detection of pregnancy rats were kept one per polypropylene cage until delivery. The offspring were kept with the dam for 4–5 weeks in housing conditions. Then, animals were kept 6–7 per cage. Animals received standard laboratory chow (Baranova et al., 1986) and tap water ad libitum.

Experiments

BrdUdr was ex tempore dissolved in distilled water and injected subcutaneously on the 1st, 3rd, 7th and 21th days of life into rats in a volume of 0.2 ml at a single dose of 3.2 mg per animal (group 2). Some rats born at the same time were kept intact and served as controls (group 1). At the age of 3 months some male and female rats neonatally exposed to BrdUrd were put together (1 male : 2–3 females) for a week for mating and conception of the offspring (group 3). The offspring conceived did not undergo any special influences. Starting from the age of 12, 15, 18 and 21 months, vaginal smears were cytologically stud-

TABLE 1

LIFE SPAN AND PARAMETERS OF AGING IN RATS NEONATALLY EXPOSED TO BrdUrd AND IN THEIR OFFSPRING (F_1)

Group	Treatment	Number of rats	Mean life span (days)		Maximum life span (days)	Constants of aging rate *	
			All rats	Tumor-free rats		$\alpha \times 10^{-3}$	$R \times 10^{-3}$
Males							
1	Control	71	779 ± 16.1	787 ± 15.5	1018	4.9 ± 0.04	6.1 ± 0.32
2	BrdUrd	85	533 ± 18.6 [a]	450 ± 21.2 [a]	955	3.4 ± 0.12 [a]	48.3 ± 0.83 [a]
3	F_1	42	708 ± 22.3 [bd]	559 ± 35.5 [ad]	903	4.6 ± 0.33 [d]	19.4 ± 0.26 [ad]
Females							
1	Control	79	722 ± 15.0	708 ± 15.0	1013	4.2 ± 0.34	7.4 ± 0.36
2	BrdUrd	107	569 ± 15.0 [a]	500 ± 14.8 [a]	853	4.3 ± 0.03	24.3 ± 0.77 [a]
3	F_1	46	670 ± 18.6 [cd]	596 ± 38.1 [bd]	879	6.1 ± 0.26 [ad]	8.4 ± 0.16 [cd]

* Constants were calculated according to Gompertz' equation: $R = R_0 \cdot (\exp. \alpha t)$ (Gavrilov and Gavrilova, 1991).
[a,b,c] The difference with the corresponding parameter in the sex-matched control is significant: [a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.05$.
[d] The difference with the corresponding parameter for the group exposed to BrdUrd is significant: $p < 0.05$.

ied daily for 30 days in 27 randomly selected females from group 1, 26 rats from group 2 and in 39 rats from group 3. Animals remained under observation until natural death or until they were killed when moribund.

Pathohistological examination

Mortality was registered in rats on days 1, 3, 7 and 21, at the age of 1, 2 and 3 months and further on until their natural deaths. Developmental stigmas in the animals were registered as well.

All animals that died or were killed were autopsied. The tumors found at necropsy were evaluated as 'fatal' or 'incidental' according to the IARC recommendations (Gart et al., 1989). At the autopsy the liver, kidneys, spleen and all macroscopically abnormal organs were fixed in 10% neutral formalin. Routine histological treatment of 5–7 μm thick sections was followed by

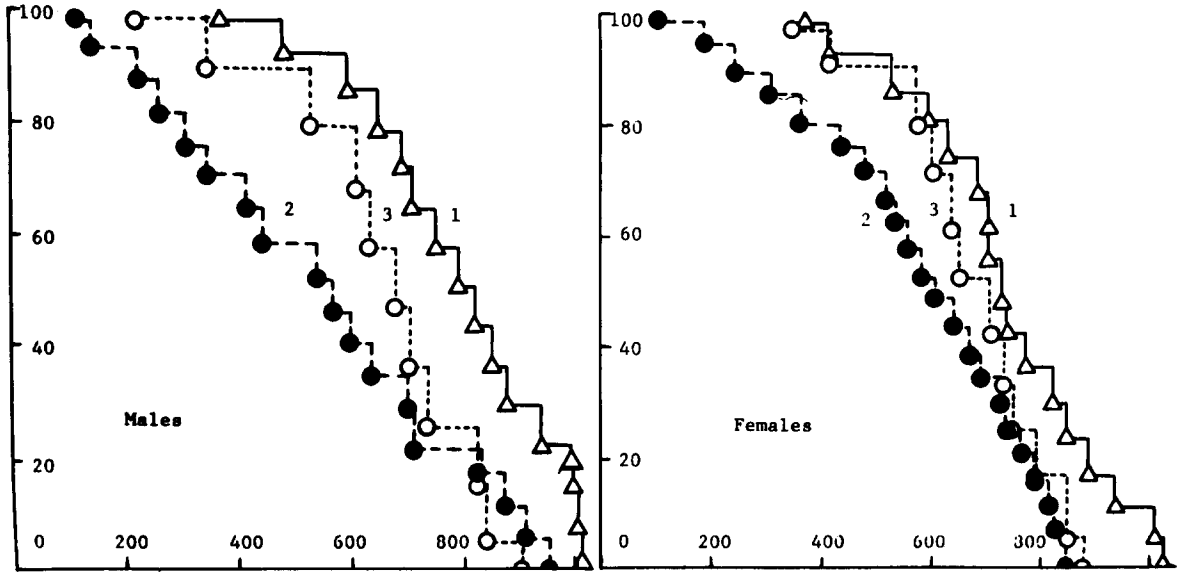

Fig. 1. Survival curves of rats exposed neonatally to BrdUrd and of their offspring (F_1). Ordinate, % of rats; abscissa, age in days. 1, control; 2, BrdUrd; 3, F_1. Symbols represent every fifth animal.

staining with hematoxylin and eosin and subsequent microscopic examination. The tumors were classified according to the IARC classification (Turusov and Mohr, 1990).

Statistics

Experimental results were statistically processed according to the IARC recommendations (Gart et al., 1989). The statistical significance of any apparent increases in carcinogenic effect with regard to BrdUrd was assessed by combine the test for fatal and incidental tumors (Gart et al., 1989). In other places, Student's *t*-test and Fischer's exact test for equality of proportions were used as appropriate (Goubler, 1978). An IBM PC/AT computer (USA) was employed for statistical processing of the data.

Results

Effect of BrdUrd on survival of the rats

Early postnatal injections of BrdUrd to rats of the parental generation led to a considerable reduction in the number of rats per litter in the offspring: 7.7 ± 0.4 versus 10.7 ± 1.0 in controls ($p < 0.05$), and to an increase in the number of stillborn rats: 12.7% versus 3.8% in controls ($p < 0.05$).

Administration of BrdUrd in early life was followed by a significant decrease of the mean and maximum life spans of male and female rats and their offspring (Table 1). A significant shift to the left of the survival curves was observed in groups of male and female rats neonatally exposed to BrdUrd in comparison to untreated animals (Fig. 1).

Effect of BrdUrd on non-tumor pathology in the rats

A variety of developmental stigmas was observed in rats of group 3: the absence of front limbs, microbrachilia, microphthalmia, short or needle-like tail and diffuse obesity. All these rats died before the age of 6–9 months. The major hereditary pathology was the absence of fingers on the front limbs. Finger absence was registered in 36.6% of males and in 31.1% of females. No stigmas were observed in groups 1 and 2.

Micro- and macrocysts of kidney convoluted tubules lined with eosinophilic epithelial cells were observed in 22.4% of BrdUrd-treated males and 46.7% females in comparison to 5.0% and 2.5% in control animals, respectively ($p < 0.001$). Cystic pathology in kidney was observed in 14.9% of F_1 males and in 13.2% of F_1 females. Cases of a decrease in relative size of liver accompanied by histologically manifested spongiosis, cases of a decrease of spleen size accompanied by declines in the size of follicles and by the fibrosis of stroma, and cases of atrophy of the testicles were

TABLE 2

TUMOR INCIDENCE AND SURVIVAL OF TUMOR-BEARING RATS EXPOSED NEONATALLY TO BdrUrd AND OF THEIR OFFSPRING (F_1)

Group	Treatment	Effective number of rats *	Total tumors			Malignant tumors		
			Number of rats	Mean survival (days)	Number of tumors	Number of rats	Mean survival (days)	Number of tumors
Males								
1	Control	71	15 (21%)	782 ± 48.1	20	4 (6%)	902 ± 43.5	4
2	BrdUrd	85	25 (33%)	688 ± 42.1	35	12 (16%)	579 ± 58.5 [b]	12
3	F_1	42	23 (55%) [a]	715 ± 19.3	43	11 (26%) [c]	703 ± 38.6 [c]	11
Females								
1	Control	79	35 (44%)	736 ± 26.7	63	5 (6%)	846 ± 110.7	5
2	BrdUrd	107	58 (56%)	622 ± 20.5 [a]	89	15 (14%) [d]	578 ± 41.3 [d]	15
3	F_1	46	38 (83%) [a]	680 ± 19.3	70	11 (24%)	512 ± 45.0 [d]	12

* The number of rats surviving until the discovery of the first tumor in the experiment (males and females separately).
[a,b,c,d] The difference with the corresponding parameters in the sex-matched control is significant: [a] $p < 0.001$; [b] $p < 0.002$; [c] $p < 0.02$; [d] $p < 0.05$ (Fischer's exact test or Student's *t*-test).

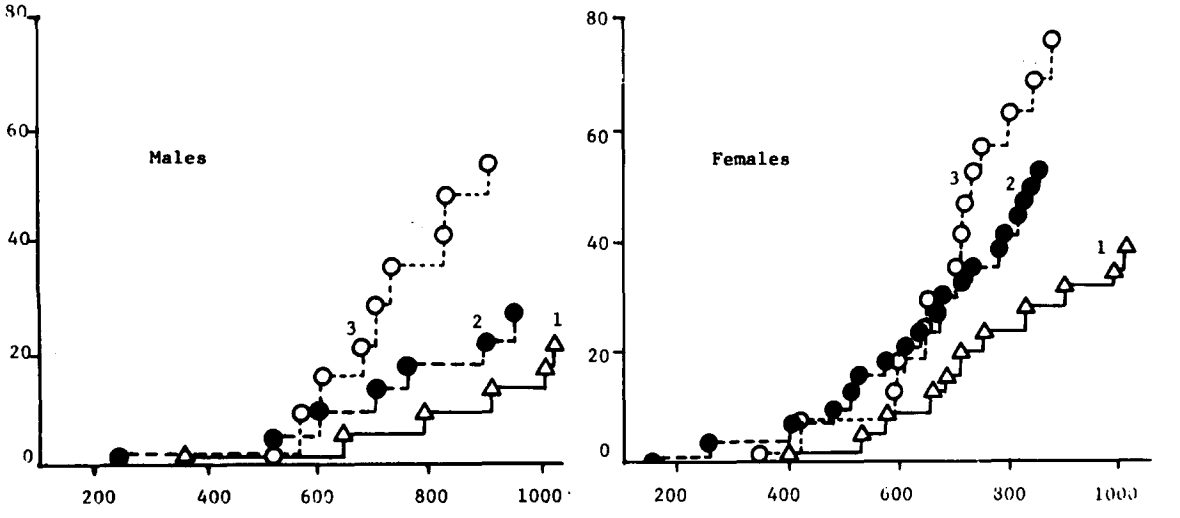

Fig. 2. Total tumor yield curves in rats exposed neonatally to BrdUrd and in their offspring (F_1). Ordinate, % of tumor-bearing rats; abscissa, age in days. 1, control; 2, BrdUrd; 3, F_1. Symbols represent every third tumor-bearing animal.

registered in rats exposed to BrdUrd. Similar phenomena were sometimes observed in F_1 rats.

Effect of BrdUrd on tumor pathology in rats and their offspring

The data on the tumor incidence and survival of tumor-bearing rats in groups 1–3 are given in Table 2, Figs. 2 and 3. Both total and malignant tumor incidence in male progeny of rats exposed neonatally to BrdUrd (group 3) were significantly increased (almost 3 times) in comparison to controls ($p < 0.05$).

The results of histological examination of tumors discovered in males are presented in Table 3. Testicular tumors (Leydigomas) and kidney tumors were observed in males exposed to Brd-

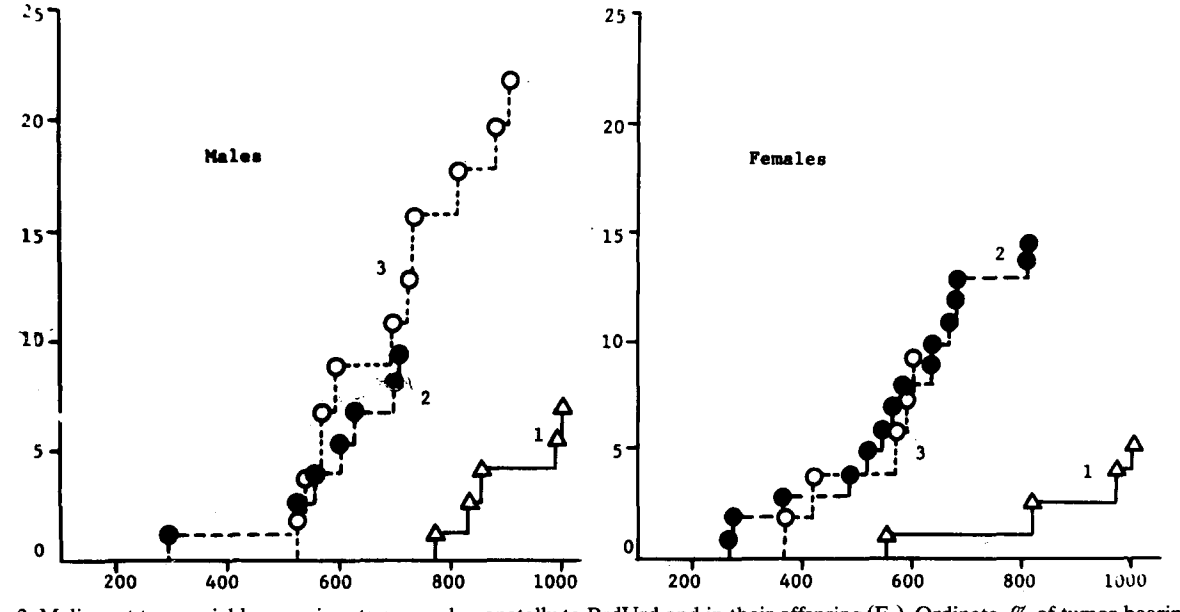

Fig. 3. Malignant tumor yield curves in rats exposed neonatally to BrdUrd and in their offspring (F_1). Ordinate, % of tumor-bearing rats; abscissa, age in days. 1, control; 2, BrdUrd; 3, F_1. Symbols represent every animal bearing a malignant tumor.

TABLE 3

TUMOR LOCALIZATION AND TYPE IN MALE RATS EXPOSED NEONATALLY TO BrdUrd AND IN THEIR MALE OFFSPRING

Tumor site	Tumor type	Group 1 (Control)	Group 2 (BrdUrd)	Group 3 (F_1)
Pituitary	Adenoma	9	4	8
	Adenocarcinoma	–	1	–
Thyroid	Adenoma	3	7 (5)	12 (10)
	Carcinoma	–	–	1
Testis	Leydigoma	–	10 (9)	8 (6)
Prostate	Adenoma	–	1	–
	Adenocarcinoma	–	1	1
Hemopoietic system	Myeloleukemia	–	–	1
	Lympholeukemia	1	2	3
	Histiocytic sarcoma	–	–	1
Thymus	Malignant thymoma	1	2	–
Kidney	Adenoma	1	–	–
	Carcinoma	1	–	–
	Mesenchymal tumor	–	3	–
Adrenal gland	Cortical adenoma	–	–	1
	Pheochromocytoma	–	–	1
Lung	Adenoma	1	–	–
Mammary gland	Fibroma and fibroadenoma	2	1	1
Colon	Adenocarcinoma	–	2	–
Soft tissues	Fibroma	–	–	1
	Malignant fibrous histiocytoma	1	2	1
Bone	Chondrosarcoma	–	–	1
	Osteosarcoma	–	–	1
Nervous system	Malignant schwannoma	–	–	1
Number of tumors				
Subtotal:	Benign	16	23	32
	Malignant	4	12	11
Total		20	35	43

Numbers in parentheses are the numbers of rats bearing the respective tumors.

Urd and never in control males. At the same time, in male F_1 progeny of rats exposed neonatally to BrdUrd (group 3) a significant increase in pituitary tumor incidence in comparison to control was found ($p < 0.002$), as well as in thyroid tumor incidence ($p < 0.001$). The incidence of Leydigomas reached 14% in group 3 versus 0% in control group 1. Moreover, a tendency of an increase in hemopoietic system malignancies was observed in group 3 in comparison to group 1. It is worth noting that in males of group 3 the number of rats bearing more than one tumor was significantly increased in comparison to group 1 or 2 (29%; 7% and 12%, respectively, $p < 0.002$).

Total tumor yield curves for males of groups 2 and 3 were substantially shifted to the left in comparison to the control (Fig. 2). The mean survival of malignant tumor-bearing males in groups 2 and 3 was significantly decreased in comparison to controls (Table 2) and the shift to the left of the malignant tumor yield curve for males from groups 2 and 3 in comparison to controls was clearly expressed (Fig. 3).

Early postnatal treatment with BrdUrd was followed by significant increases in both total and malignant tumor incidence in females and in their female progeny (Table 4, Figs. 2 and 3). In female F_1 progeny (group 3) a significant increase in

pituitary tumor incidence in comparison to controls was found ($p < 0.02$) as well as in thyroid tumor incidence ($p < 0.02$).

The mean survival time of total tumor-bearing females as well as of malignant tumor-bearing females was significantly reduced in group 2 in comparison to controls, whereas only the mean survival time of malignant tumor-bearing F_1 females (group 3) was decreased as compared with group 1 (Table 2, Figs. 2 and 3).

Effect of BrdUrd on estrus function of female rats

Daily cytological study of the vaginal smears showed an age-related decrease in the number of animals with regular estrus cycles in all groups under observation. Thus, among monitored control rats (group 1) estrus cycles were found to be regular in 74.1% cases at the age of 12–13 months and in 36.8% at the age of 21 months. In females exposed to BrdUrd (group 2) the corresponding values were 42.3% and 30.0% and in F_1 rats (group 3) 51.3% and 26.3% (Table 5).

The results of daily cytological study of vaginal smears of female rats exposed and non-exposed to BrdUrd were analyzed in terms of the ratio of phases of the estrus cycle (estrus : proestrus + metaestrus : diestrus) and to the length of the estrus cycle. Fully normal estrus cycles (when all

TABLE 4

TUMOR LOCALIZATION AND TYPE IN FEMALE RATS EXPOSED NEONATALLY TO BrdUrd AND IN THEIR FEMALE OFFSPRING

Tumor site	Tumor type	Group 1 (Control)	Group 2 (BrdUrd)	Group 3 (F_1)
Pituitary	Adenoma	18	10	24
	Adenocarcinoma	–	–	1
Thyroid	Adenoma	3	11	5
	Adenocarcinoma	–	–	1
Mammary gland	Fibroma and fibroadenoma	33 (23)	34 (27)	20 (14)
	Adenocarcinoma	–	–	1
	Malignant fibrous histiocytoma	–	1	–
Ovary	Thecoma	1	1	1
	Malignant thecoma	–	–	1
Uterus	Endometrial polyp	–	3	–
	Adenocarcinoma	1	1	2
	Adenosarcoma	–	1	–
Hemopoietic system	Lympholeukemia	1	3	3
	Myeloleukemia	1	1	1
Thymus	Thymoma	–	–	1
	Malignant thymoma	1	–	–
Kidney	Carcinoma	–	–	1
	Mesenchymal tumor	1	3	–
Liver	Cholangioma	–	1	–
Pancreas	Adenocarcinoma	–	1	–
Stomach	Polyp	–	1	–
Colon	Adenocarcinoma	–	1	–
Adrenal gland	Cortical adenoma	3	–	4
	Pheochromocytoma	–	–	2
Zymbal gland	Squamous cell carcinoma	–	1	–
Soft tissues	Benign	–	3	1
	Malignant	–	2	1
Number of tumors				
Subtotal:	Benign	58	74	58
	Malignant	5	15	12
Total:		63	89	70

Numbers in parentheses are the numbers of rats bearing the respective tumors.

TABLE 5

ESTRUS FUNCTION PARAMETERS IN FEMALE RATS EXPOSED NEONATALLY TO BrdUrd AND IN THEIR FEMALE OFFSPRING

Parameter	Group 1 (Control)	Group 2 (BrdUrd)	Group 3 (F_1)
Age: 12–13 months			
Number of rats monitored	27	26	39
regularly cycling (%)	74.1	42.3 [a]	51.3 [a]
non-regularly cycling (%)	14.8	23.1	2.5
with persistent estrus + anestrus (%)	11.1	34.6 [a]	46.2 [a]
Estrus cycle length (days)	5.1 ± 0.20	6.1 ± 0.22 [a]	5.5 ± 0.33
Age: 15–16 months			
Number of rats monitored	26	23	34
regularly cycling (%)	61.5	39.1 [a]	47.1 [a]
non-regularly cycling (%)	11.6	8.7	0.0
with persistent estrus + anestrus (%)	26.9	26.9	52.9 [a]
Estrus cycle length (days)	6.2 ± 0.24	7.1 ± 0.33 [a]	6.8 ± 0.39
Age: 18–19 months			
Number of rats monitored	23	20	32
regularly cycling (%)	34.8	25.0	21.9
non-regularly cycling (%)	30.4	15.0	12.5
with persistent estrus + anestrus (%)	34.8	60.0 [a]	65.6 [a]
Estrus cycle length (days)	6.4 ± 0.22	7.2 ± 0.75	6.6 ± 0.26
Age: 21–22 months			
Number of rats monitored	19	10	19
regularly cycling (%)	36.8	30.0	26.3
non-regularly cycling (%)	15.9	0.0	15.8
with persistent estrus + anestrus (%)	47.4	70.0 [a]	57.9 [a]
Estrus cycle length (days)	7.4 ± 1.12	9.7 ± 0.48	10.2 ± 0.66 [a]

[a] The difference with the corresponding parameter for the control group is significant, $p < 0.05$.

phases were detected) were selected for calculations. An age-related increase in the diestrus phase in comparison to estrus or proestrus + metaestrus phases we observed in all studied groups. The mean length of the estrus cycle increased with advancing age in all studied groups. In F_1 females (group 3) this parameter was similar to that in controls between the age of 12 and 18 months, and was significantly increased in comparison to controls at the age of 21 months.

Discussion

It was shown that the exposure of pregnant female C3H-MF mice to BrdUrd was followed by the development of anormalities in 55% of offspring, mainly presented by polydactylia of the hind limbs (Di Paolo, 1964). We observed the absence of fingers on the front limbs in one third of the offspring of BrdUrd-exposed rats. This malformation seems to be not a result of metabolic disturbances, as Di Paolo (1964) supposed, but rather to indicate the tropism of BrdUrd to certain sites in the genome. Micro- and macrocystosis in kidneys was developed in both the parental generation and its offspring. This phenomenon might be a result of BrdUrd-induced specific damages in DNA leading to disturbances in differentiation of kidney tissues.

Early postnatal exposure to BrdUrd was followed by a considerable shift to the left of the survival curves of rats of the parental generation and of their offspring (Fig. 1) and by an acceleration of their aging rate (Table 1). These results are in accordance with previous observations on the life span-reducing potential of BrdUrd in rats

(Craddock, 1981; Anisimov and Osipova, 1992). The aging rate and R_0 calculated for F_1 rats were also increased in comparison to controls (group 1), but their values were lower than for rats exposed to BrdUrd (group 2) (Table 1).

The toxic effect of BrdUrd in the parental generation is probably due to one of its degradation products, bromine. Unlike the rats which were given BrdUrd, the early deaths of their progeny may depend on the development of some inherent mutations, incompatible with further life. In our previous work (Anisimov and Osipova, 1992) we have discussed the question of whether the life span reduction induced by BrdUrd depends on its specific effect on the aging process or is a consequence of toxicity of the agent. The results of the present experiment suggested that neonatal exposure to BrdUrd was followed by some toxic effects: delay in body weight gain, delay in the age of opening of the vagina and an age-related decrease in the length of the estrus cycle during the period of maturation, a decrease in the mean body weight during the whole period of observation and a decrease in relative size of some organs in rats treated neonatally with BrdUrd in comparison with those in the untreated controls. At the same time some signs of accelerated aging in rats neonatally exposed to BrdUrd were demonstrated. Thus, the calculation according to Gompertz' equation of the mortality parameters for both untreated rats and rats neonatally exposed to BrdUrd that survived to an age of 3 months revealed an acceleration of the aging rate under the influence of the thymidine analogue.

The results of monitoring the estrus function suggested an acceleration of the natural age-related switching-off of reproductive function in female rats neonatally exposed to BrdUrd. This process was gradual and included not only earlier increases in the incidence of estrus cycle disturbances (persistent estrus and/or anestrus) in BrdUrd-treated rats in comparison to untreated ones, but also an earlier increase in the mean length of normal estrus cycles than in control. We observed an increase in pituitary and thyroid tumor incidence in rats exposed to BrdUrd in comparison to controls. Taking into consideration a basic role of the neuroendocrine axis in the aging process (Dilman and Dean, 1992), these findings support the suggestion of an acceleration of aging in rats treated with BrdUrd (Anisimov and Osipova, 1992). The exposure of mice and rats to some other genotoxic and carcinogenic agents was also followed by signs of accelerated aging (for review see Anisimov, 1987).

It is worth noting that in the F_1 offspring of rats exposed neonatally to BrdUrd an acceleration of both the aging rate and the aging of reproductive function was also observed. A similarity of patterns of changes in aging rate and in age-related disturbances in the function of the reproductive system in the parental and F_1 generations suggests a primary role of BrdUrd-induced DNA damages in both these phenomena. It is clear that this suggestion needs more evidence. Also it is important to have some additional data on age-related changes in male rats exposed to BrdUrd. For example, registration of the time of the prepubertal peak of serum testosterone and evaluation of the dynamics of age-related changes in testosterone levels in males may give valuable information on this matter. Special attention must be given to a quantitation of DNA sequence changes in the organism, to a characterization of age-related organ- and tissue-specific variations in spectra of DNA sequences, and to a search for a correlation between these spectra of DNA defects and age-related pathology, including cancer (Vijg, 1990).

In the present study as well as in earlier reports (Napalkov et al., 1989a; Anisimov and Osipova, 1992) the neonatal administration of BrdUrd was shown to be followed by a slight increase in total tumor incidence and by a shortening of the tumor latency. The majority of developed tumors was presented by neoplasias characteristic for the rat strain used. However, in rats receiving BrdUrd we observed the development of tumors of gonads, kidney, uterus and some other locations which are infrequent in untreated rats (Anisimov et al., 1989). It is worth noting that the spectrum of tumors developing in the progeny of rats exposed neonatally to BrdUrd is similar to that in the parental generation. This observation suggests a similarity in the mechanisms of carcinogenesis in rats directly exposed to the nucleoside analogue and in their offspring through

germ-line DNA damages and provides additional evidence that a sole perturbation of DNA contributed substantially not only to the induction of cell transformation in vitro (Barrett et al., 1978), but also to carcinogenesis in vivo (Napalkov et al., 1989a).

At the same time, the molecular mechanisms of the observed phenomena are not yet clear. The presence of 5-bromouracil in DNA results in a very low probability of miscoding (Davidson et al., 1988; Kaufman, 1988). However, 5-bromouracil is not a substrate for DNA repair enzymes and thus persists over a long period in some tissues (Likhachev et al., 1983a; Ward et al., 1991) leading primarily to GC → AT transitions (Davidson et al., 1988; Kaufman, 1988). The results of the present experiments as well as the observation of an increase in the incidence of chromosome aberrations in lymphocytes (Anisimov and Osipova, 1992) and of point mutations in the K-*ras* protooncogene in mesenchymal kidney tumors induced by neonatal exposure to BrdUrd (Calvert et al., 1992) suggest that in vivo mutagenesis was induced by administration of the agent in early life.

The variety of random DNA damages induced by BrdUrd causing instability of the cell genome may be an initial step in carcinogenesis. However, there is evidence that some other factors (for example, factors affecting birth weight or placental size) have serious consequences for longevity and tumorigenesis in offspring (Berstein, 1988; Napalkov et al., 1989b). In any case, our results provide new evidence to support the hypothesis of vertical transmission of the carcinogenic effect of exogenic agents through generations.

Acknowledgements

The authors are grateful to Prof. N.P. Napalkov and Dr. L. Tomatis for their interest in this work, Dr. A.J. Likhachev for discussion and valuable suggestions, O. Gvardina, I. Mikhailova and E. Solomatina for technical assistance. This work was supported in part by a grant from the Ministry of Science of the Russian Federation.

References

Anisimov, V.N. (1987) Carcinogens as accelerators of aging. In: V.N. Anisimov (Ed.), Carcinogenesis and Aging, Vol. 2, CRC Press, Boca Raton, FL, pp. 37–55.

Anisimov, V.N., and G.Yu. Osipova (1992) Effect of neonatal exposure to 5-bromo-2′-deoxyuridine on life span, estrus function and tumor development in rats – an argument in favor of the mutation theory of aging? Mutation Res., 275, 97–110.

Anisimov, V.N., G.B. Pliss, M.G. Iogannsen, I.G. Popovich, K.P. Romanov, A.S. Monakhov and T.K. Averyanova (1989) Spontaneous tumors in outbred LIO rats, J. Exp. Clin. Cancer Res., 8, 254–262.

Anisimov, V.N., I.E. Vorobtsova and L.M. Aliyakparova (1992) X-ray irradiation of male mice before mating increases the susceptibility of progeny in two generations to the promoter of skin carcinogenesis 12-*O*-tetradecanoylphorbol-13-acetate, In: T. Sugahra, L.A. Sagan and T. Aoyama (Eds.), Low Dose Irradiation and Biological Defence Mechanisms, Excerpta Medica, Amsterdam, pp. 159–162.

Baranova, L.N., K.P. Romanov and V.A. Yamshanov (1986) Study of levels of benzo[*a*]pyrene and *N*-nitrosamines in the food of laboratory animals. Vopr. Onkol., 5, 54–57.

Barrett, J.C., T. Tsutsui and P.O.P. Ts'O (1978) Neoplastic transformation induced by a direct perturbation of DNA, Nature, 274, 229–232.

Berstein, L.M. (1988) Newborn macrosomy and cancer, Adv. Cancer Res., 50, 231–278.

Calvert, R.J., G.S. Buzard, V.N. Anisimov and J.M. Rice (1992) K-ras codon 12 point mutations in bromodeoxyuridine-induced rat renal mesenchymal tumors, Abstracts of the 8th Annual Meeting on Oncogenes, 23–27 June 1992, Hood College, MD, p. 309.

Christov, K., K.L. Chew, B.-M. Ljung, F.W. Waldman, L. Duarte, W.H. Goodson, H.S. Smith and B.H. Mayall (1991) Proliferation of normal breast epithelial cells as shown by in vivo labeling with bromodeoxyuridine, Am. J. Pathol., 138, 1371–1380.

Craddock, V.M. (1981) Shortening of the life span caused by administration of 5-bromodeoxyuridine to neonatal rats, Chem.-Biol. Interact., 35, 139–144.

Davidson, R.L., P. Broeker and C.R. Ashman (1988) DNA base sequence changes and sequence specificity of bromodeoxyuridine-induced mutations in mammalian cells, Proc. Natl. Acad. Sci. USA, 85, 4405–4410.

Dilman, V.M., and W. Dean (1992) The Neuroendocrine Theory of Aging and Degenerative Disease, Center for Bio-Gerontology, Pensacola, FL.

Di Paolo, S.A. (1964) Polydactylism in the offspring of mice injected with 5-bromodeoxyuridine, Science, 145, 501–503.

Gardner, J.S., M.P. Snee and A.J. Hall (1990) Results of a case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria, Br. Med. J., 300, 423–429.

Gart, J.J., D. Krewsky, P.N. Lee, R.E. Tarone and J. Wahrendorf (1986) Statistical Methods in Cancer Research. Vol.

III: The Design and Analysis of Long-Term Animal Experiments, IARC Sci. Publ. No. 79, International Agency for Research on Cancer, Lyon.

Gavrilov, L.A., and N.S. Gavrilova (1991) The Biology of Life Span: A Quantitative Approach, Harwood Academic Publishers, Chur.

Goubler, E.V. (1978) Calculating Methods of Analysis and Identification of Pathological Processes, Meditsina, Moscow.

Kaufman, E.R. (1988) The role of deoxyribonucleotide metabolism in 5-bromo-2′-deoxyuridine mutagenesis in mammalian cells, Mutation Res., 200, 149–155.

Kirk, D.L., G.J. Baran, J.F. Harper, R.J. Huskey, K.S. Huson and N. Zagris (1987) Stage-specific hypermutability of the reg A locus of Volvox, a gene regulating the germ-soma dichotomy, Cell, 48, 11–24.

Lewis, J.G. and J.A. Swenberg (1982) The effect of 1,2-dimethylhydrazine and diethylnitrosamine on cell proliferation and unscheduled DNA synthesis in target and nontarget cell populations in rat liver following chronic administration, Cancer Res., 42, 89–92.

Lieber, M.M., and G.J. Todaro (1973) Spontaneous and induced production of endogenous type-C virus from a clonal line of spontaneously transformed BALB/3T3, Int. J. Cancer, 11, 616–627.

Likhachev, A.J., L. Tomatis and G.P. Margison (1983) Incorporation and persistence of 5-bromodeoxyuridine in newborn rat tissue DNA, Chem.-Biol. Interact., 46, 31–38.

Lindahl, T. (1982) DNA repair enzymes, Annu. Rev. Biochem., 51, 61–87.

Miller, M.A., C.M. Mazewski, N. Yousuf, Y. Sheikh, L.M. White, G.A. Yanik, D.M. Yams, B.C. Lumpkin and A. Raza (1991) Simultaneous immunohistochemical detection of IudR and BrdU infused intravenously to cancer patients. J. Histochem. Cytochem., 39, 407–414.

Morris, S.M. (1991) The genetic toxicology of 5-bromodeoxyuridine in mammalian cells, Mutation Res., 258, 161–188.

Morris, S.M., L.J. McGarrity, O.E. Domon, W.G. Hinson and P.L. Kodell (1989) Flow cytometric analysis of bromodeoxyuridine-induced inhibition of cell proliferation in the human tetratocarcinoma-derived cell line, P3, Environ. Mol. Mutagen., 14, 107–112.

Napalkov, N.P., V.N. Anisimov, A.J. Likhachev and L. Tomatis (1989a) 5-Bromodeoxyuridine-induced carcinogenesis and its modification by persistent estrus syndrome, unilateral nephrectomy, and X-irradiation in rats, Cancer Res., 49, 318–323.

Napalkov, N.P., J.M. Rice, L. Tomatis and H. Yamasaki (Eds.) (1989b) Perinatal and Multigeneration Carcinogenesis, IARC Sci. Publ. No. 96, International Agency for Research on Cancer, Lyon.

Nomura, T. (1991) Of mice and men?, Nature, 345, 671.

Ruffolo, P.R., and V.H. Ferm (1965) The embryonal and teratogenic effects of 5-bromodeoxyuridine in the pregnant hamster, Lab. Invest., 14, 1547–1553.

Stockdale, F., K. Okasaki, M. Nameroff and M. Holzer (1964) 5-Bromodeoxyuridine: effect on myogenesis in vitro, Science, 146, 533–535.

Szybalsky, W. (1974) X-ray sensitization by halopyrimidines, Cancer Chemother. Rep., 58, 539–557.

Tapscott, S.J., A.B. Lassar, R.L. Davis and H. Weintraub (1989) 5-Bromo-2′-deoxyuridine blocks myogenesis by extinguishing expression of MyoDi, Science, 245, 532–534.

Tomatis, L., J.R.P. Cabral, A.J. Likhachev and V.I. Ponomarkov (1981) Increased cancer incidence in the progeny of male rats exposed to ethylnitrosourea before mating, Int. J. Cancer, 28, 475–478.

Trautner, T.A., M.N. Swartz and A. Kornberg (1962) Enzymatic synthesis of deoxyribonucleic acid. X. Influence of bromouracil substitution on replication. Proc. Natl. Acad. Sci. USA, 48, 449–455.

Turusov, V.S., and U. Mohr (Eds.) (1990) Pathology of Tumours in Laboratory Animals. Vol. I: Tumours of the Rat, 2nd edn. IARC Sci. Publ. No. 99, International Agency for Research on Cancer, Lyon.

Turusov, V.S., L. Tomatis, R. Cabral, E. Cardis and N.F. Tiutiunnik (1988) Transzygotic effect of nitrosourea in rats, Exp. Onkol., 10, 25–28.

Vijg, J. (1990) DNA sequence changes in aging: how frequent, how important?, Aging, 2, 105–123.

Ward, J.M., J.R. Henneman, G.Y. Osipova and V.N. Anisimov (1991) Persistence of 5-bromo-2′-deoxyuridine in tissues of rats after exposure in early life, Toxicology, 70, 345–352.

Weghorst, C.M., J.R. Henneman and J.M. Ward (1991) Dose response of hepatic and DNA synthesis rates to continuous exposure of bromodeoxyuridine (BrdU) via slow-release pellets or osmotic minipumps in male B6C3F1 mice, J. Histochem. Cytochem., 39, 177–182.