

Acta Medica Okayama

Volume 37, Issue 1

1983

Article 5

FEBRUARY 1983

Effect of oncogenic nitrosourea on intraocular tumor induction by adenovirus in rats.

Shoji Kobayashi*

Katsuo Ogawa†

*Kagawa Medical School,

†Okayama University,

Effect of oncogenic nitrosourea on intraocular tumor induction by adenovirus in rats.*

Shoji Kobayashi and Katsuo Ogawa

Abstract

Interference of oncogenic N-nitrosourea in intraocular tumor induction by human adenovirus type 12 in rats was examined. Transplacental administration of methyl nitrosourea to rat embryo reduced significantly the latent period of the intraocular adenovirus tumor in each animal whereas in groups preadministered with ethyl nitrosourea the decrease in the latent period showed marked individual variation. Preadministration of N-nitrosourea caused little change in the morphology and incidence of adenovirus tumors. The histological picture of adenovirus induced intraocular tumors which developed in each group of rats was that of retinoblastoma-like tumor identical to the tumor induced by single virus injection.

KEYWORDS: adenovirus type 12, nitrosourea, brain tumor, latent period, retin-oblastoma-like tumor

*PMID: 6846051 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 37, (1), 45-49 (1983)

EFFECT OF ONCOGENIC NITROSOUREA ON INTRAOCULAR TUMOR INDUCTION BY ADENOVIRUS IN RATS

Shoji KOBAYASHI* and Katsuo OGAWA

* Department of Pathology, Kagawa Medical School, Kagawa 761-07 and Department of Pathology, Okayama University Medical School, Okayama 700, Japan

Received August 2, 1982

Abstract. Interference of oncogenic N-nitrosoarea in intraocular tumor induction by human adenovirus type 12 in rats was examined. Transplacental administration of methylnitrosoarea to rat embryo reduced significantly the latent period of the intraocular adenovirus tumor in each animal whereas in groups preadministered with ethylnitrosoarea the decrease in the latent period showed marked individual variation. Preadministration of N-nitrosoarea caused little change in the morphology and incidence of adenovirus tumors. The histological picture of adenovirus induced intraocular tumors which developed in each group of rats was that of retinoblastoma-like tumor identical to the tumor induced by single virus injection.

Key words : adenovirus type 12, nitrosoarea, brain tumor, latent period, retinoblastoma-like tumor.

Transplacental administration of oncogenic nitrosoarea compounds to the rat fetus in the latter half of pregnancy induces a high incidence of adult type neuroepithelial tumors (1). On the other hand, it is known that direct intracranial inoculation of human adenovirus type 12 (Ad12) into newborn rats, hamsters and mice produces undifferentiated infantile type brain tumors (2), and that intra-ocular inoculation produces retinoblastoma-like tumors in mice and rats (3, 4).

In the present report, N-ethyl-N-nitrosoarea (ENU) and methyl-nitrosoarea (MNU) were administered to rats during late pregnancy. The newborns from these animals were inoculated intra-ocularly with Ad12 and the effect of two types of neurotropic chemical carcinogens on adenovirus oncogenesis was investigated.

MATERIALS AND METHODS

Animal experiments. CD strain rats (Sprague-Dawley descended) were divided into three groups on the 21st day of pregnancy. Group 1 rats were given ENU intravenously at a dose of 20 mg/kg. Group 2 rats were given MNU intravenously at a dose of 10 mg/kg. The administration of nitrosoarea was not performed in Group 3 rats as a control.

Ad12 fluid ($10^{2.5}$ TCID₅₀/0.1 ml HeLa cells, an injection volume of 10 μ l) was injected

into the left eye of the newborn from the mothers of groups 2 and 3 rats within 24 h of being born. The newborn of group 1 rats were injected with Ad12 fluid into both the left and right eyes (10 μ l into each eye).

The animals were reared on tap water and solid food pellets (MF, Oriental Co., Tokyo). Animals with definite development of tumors were sacrificed and given a full systemic autopsy with histological investigation of all of the brain and tumor tissue. The total observation time was 300 days. Animals which survived to 300 days were killed and the existence of tumors was searched for macroscopically. Tissue specimens of the brain were made and examined.

Virus fluid. Human adenovirus type 12 (Huic strain) was propagated in HeLa cells. The HeLa cells were maintained with Eagle's MEM supplemented with 2 % calf serum. Virus fluid was harvested and aliquots were stored at -70°.

Nitroso-compounds. The nitroso-compound ENU was kindly donated by the Japan Institute for Research on Light Sensitive Dye (Nihon Kanko Shikiso Kenkyusho), Okayama. MNU was purchased from K & K Chemicals Co. (USA).

RESULTS

In group 1 rats, tumors developed in 24 of 48 animals. Of these 24 rats, 12 developed Ad12-induced tumors and 10 had tumors induced by ENU. Two rats developed both Ad12 and ENU induced tumors. In 8 of 12 animals Ad12-induced tumors developed in the eyeball only. Two animals developed both intra-ocular and intracranial Ad12-induced tumors. In two animals, only brain tumors developed (Table 1).

The histological picture of the Ad12-induced intra-ocular tumors which developed in each group of rats was the same as the retinoblastoma-like tumor previously reported (3); that is, the tumor cells were short spindle to ovoid cells

TABLE 1. INTERFERENCE OF ONCOGENIC N-NITROSO COMPOUNDS
ON TUMOR INDUCTION BY ADENOVIRUS IN RATS

Groups	Pre-treatment with nitrosourea mg/kg	Numbers of offspring	Inoculation of Ad 12, volume (μ l) and site	Number of animals with tumor (s)			Average latency days
				Ad 12 induced	Nitrosourea induced	Ad 12 and nitrosourea induced	
1	ENU, 20	48	10	12 * ∇	10**	2***	*160
			Both eyes	(25.0 %)	(20.8 %)		**200 ***249
2	MNU, 10	50	10	5	0	0	96
			Left eye	(10.0 %)			
3	no	43	10	4	0	0	226
			Left eye	(9.3 %)			

*, **, *** : Average latency days corresponding to each group of tumor bearing animals.

∇ : Eight of 12 animals induced ocular tumor, two of 12 induced both ocular and brain tumors, and two of 12 induced brain tumor only.

with small, chromatin rich nuclei showing prominent atypism. The tumor cells proliferated thickly and formed Horner-Wright type rosettes, although the formation of Flexner-Wintersteiner type rosettes, which are frequently seen in human retinoblastoma, was not observed. The histological picture of the Ad12-induced brain tumors was, as previously reported, that of an infantile undifferentiated brain tumor (2, 5-7). ENU tumors developed in ten animals. Multiple tumors by ENU developed in four rats. The numbers of tumors induced by ENU when listed by histological type were: malignant neurinoma of the intracranial trigeminal nerve (6), oligodendroglioma (5), isomorphous mixed glioma (2), ependymoma (1), and, as a tumor that arose apart from the nervous system, one nephroblastoma.

In the two rats that simultaneously developed Ad12 and ENU induced tumors, the Ad12-induced tumors in both animals were intra-ocular retinoblastoma-like tumors. The ENU induced tumors of these two were a cerebral oligodendroglioma in one animal, and a malignant neurinoma of the optic nerve in the orbit on the contralateral side (the side without the retinoblastoma-like tumor) in the other.

MNU induced tumors did not develop in the group 2 rats preinjected with MNU at doses which were less than the oncogenic dose. Ad12-induced retinoblastoma-like tumors developed intraocularly in 5 of these animals.

In group 2 and group 3 rats, the incidence of cancer development due to Ad12 seemed low, in contrast to that in group 1 rats, in which it seemed high. However, since virus fluid was inoculated into both eyeballs in group 1, the incidence in relation to the number of eyeballs inoculated with virus was almost the same in all three groups.

The latent period to the development of Ad12-induced tumors was markedly shorter in the group 2 rats which had been preinjected with MNU. In group 1 rats preinjected with ENU, the extent of the decrease in this latent period showed marked individual variation (Fig. 1).

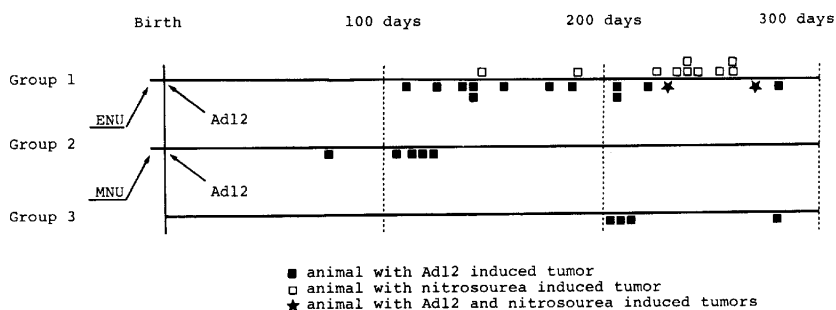


Fig. 1 Comparison of latency period for tumors induced by adenovirus and oncogenic nitrosoarea compound

DISCUSSION

Both Ad12 and nitrosourea have the same oncogenic effect causing neurogenic tumors but they differ from each other in several points. Ad12 induces extremely undifferentiated neuroectodermal tumors (infantile type brain tumors) in rats, hamsters and in some inbred mice. In contrast to this, nitrosourea mainly induces well differentiated tumors (adult type of brain tumor); moreover, although tumor incidence is high in rats, the incidence of neurogenic tumor is extremely low in mice and hamsters (8,9). In rat embryo, nitrosourea causes destruction of immature nerve cells in the early period after administration, and gives rise to marked maldevelopment of the cerebellum and retina. Ad12, however, causes hardly any destructive effects on the cells in rodents.

The oncogenic target cells in Ad12-induced brain tumors are the ependy-moglioblasts in the developing subependymal layer (7). In the eye, the target cells are undifferentiated neural cells in the retina. It was thought that nitrosourea administered transplacentally probably influences the oncogenic changes in undifferentiated target cells as caused by Ad12, and may act as a cocarcinogen in Ad12 tumorigenesis. In the present experiment, the incidence of Ad12 tumors was not affected by administration of nitrosourea in any of the groups of rats. However, the latent period was markedly affected in all groups.

In spite of the fact that the number of central nervous system tumors induced by MNU was too small to be of use, the latent period until the development of Ad12 tumors was shortened markedly by the dose of MNU preadministered to group 2 rats. This is thought to be a promotion process rather than any influence of MNU on the initiation of Ad12 induced tumors. In group 1 rats, however, the decrease in the latent period was not marked even though the dose of ENU was large enough to result in oncogenicity due to the ENU itself. The oncogenic action of nitrosourea itself, therefore, is apparently a different mechanism to that causing the shortened latent period in Ad12-induced tumors.

Reports (10-12) of nitrosourea causing abnormalities of the retina, the cerebellar granular layer, and the subependymal layer of the cerebrum would lead one to expect some variation in the histological appearance of adenovirus tumors which arise in these organs. However, this expectation was not fulfilled. The histological appearance of the Ad12-induced tumors did not vary at all after preadministration of nitrosourea.

In spite of the fact that Ad12 fluid was injected into the eyeball in group 1 rats, brain tumors developed from the Ad12 fluid. This can be interpreted as tumors being induced by virus fluid that entered the newborn brain when the needle which injected the very small eyeball of the newborn penetrated soft cranium.

In group 1 rats, there were two animals in which ENU induced tumors and Ad12 induced tumors developed simultaneously. In these rats, the two

types of tumors developed in completely separate areas with apparently no relationship at all. This indicates that the target cells of these carcinogens may have delicate differences in their spectrum and degree of cell differentiation.

Acknowledgment. This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan (N. 501052).

REFERENCES

1. Wechsler, W., Ivancovic, S., Kleihues, P., Matsumoto, S., Preussman, R., Druckey, H. and Zuelch, K.Z.: Pathology of experimental neurogenic tumors chemically induced during pre- and postnatal life. *Ann. New York Acad. Sci.* **159**, 360-408, 1969.
2. Ogawa, K.: Experimental induction of an infantile type brain tumor by adenovirus type 12. *Proc. of VII Internatl. Congress of Neuropathol.* pp. 461-468, 1974.
3. Kobayashi, S. and Mukai, N.: Retinoblastoma-like tumors induced by human adenovirus type 12 in rats. *Cancer Res.* **34**, 1646-1651, 1974.
4. Kobayashi, S., Sonobe, H., Moriwaki, K. and Ogawa, K.: Experimental model of retinoblastoma in inbred mice. *Jpn. J. Ophthalmol.* **22**, 331-337, 1978.
5. Mukai, N. and Kobayashi, S.: Human adenovirus induced medulloepitheliomatous neoplasms in Sprague-Dawley rats. *Am. J. Pathol.* **73**, 671-690, 1973.
6. Murao, T., Ohmori, H., Sonobe, H., Matsuo, K., Tsutsumi, A. and Ogawa, K.: Brain tumors induced in rats by human adenovirus type 12. *Acta Med. Okayama* **28**, 47-58, 1974.
7. Ogawa, K.: Adenovirus type 12 induced tumors -approach to experimental brain tumor-. *Tr. Soc. Pathol. Jpn.* **68**, 35-78, 1979 (in Japanese).
8. Koestner, A., Swenberg, J.A. and Wechsler, W.: Experimental tumors of the nervous system induced by resorptive N-nitrosourea compounds. ed. W.G.Bingham, In *Prog. exp. Tumor Res.* Kager, Basel, Vol. 17, 9-30, 1979.
9. Wechsler, W., Rice, J.M. and Vesselinovitch, S.D.: Transplacental and neonatal induction of neurogenic tumors in mice: comparison with related species and with human pediatric neoplasms. *Natl. Cancer Inst. Monogr.* **51**, 219-226, 1979.
10. Fujiwara, F.: Cytotoxic effects of ethylnitrosourea on central nervous system of rat embryos. *Acta Pathol. Jpn.* **30**, 375-387, 1980.
11. Wechsler, W.: Old and new concept of oncogenesis in the nervous system of man and animals. ed. W.G.Bingham, In *Prog. exp. Tumor Res.* Kager, Basel, Vol. 17, 219-278, 1979.
12. Yanagida, K.: Developmental disorder of carcinogenic nitrosourea on cerebellum and retina of hamster embryo and newborn. *J. Karyopathol.* **14**, 17-26, 1972 (in Japanese).