BIKEN JOURNAL Vol. 12, 125-126, 1969

SHORT COMMUNICATION

DEVELOPMENT OF TUMORS IN HAMSTERS BY *IN UTERO* INJECTION OF ADENOVIRUS TYPE 12 INTO FETUSES

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(Received February 28, 1969)

The importance of fetal injuries due to viral infection in the development of congenital abnormalities and malignant tumors (Bellanti et al., 1965, Kono et al., 1968, Toolan, 1960) is receiving increasing and wide spread interest.

Trentin et al. (1962) were the first to report that malignant tumors could be produced in hamsters by injection of human adenovirus type 12 at birth.

The present paper describes the development of an apparently neurogenic neoplasm in hamsters by injection of human adenovirus type 12 directly into fetuses during the later stage of pregnancy.

Adenovirus type 12 prototype strain, Huie, was propagated in HeLa cells using Earl's balanced salt solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate. This virus suspension was shown to induce tumors at a high rate (86–100%) in the intrapleural, intrapulmonary and subcutaneous regions of Syrian hamsters when 0.02 ml of preparation containing 10^3 or 10^4 times the $TCID_{50}$ was injected by the intrapulmonary route.

In the present work, pregnant hamsters were anesthetized with ether and the uterus was exposed surgically. Then 0.02 or 0.04 ml of virus preparation containing 10^3 or 10^4 times the $TCID_{50}$ was injected directly into the fetuses by various routes, as shown in Table 1. The uterus was replaced in the normal position and the abdominal wall was sutured. After this operation most hamsters completed the normal course of pregnancy, though successful delivery of babies was observed with less than half the animals (Table 1).

Eight of the 17 fetuses injected with tissue fluid only, were born normally, and no tumors developed in any of these within 150 days. Ten and 400 $TCID_{50}$ of virus were injected into 23 soy-bean sized fetuses 7 to 9 days before delivery. Of these, 15 were born, but none developed tumors or other abnormalities. Tumors were not induced by 107 days after birth when 32 fetuses were inoculated with a dose of 20 $TCID_{50}$ of virus via the amniotic cavity. Of 31 fetuses injected intracranially and 20 injected intraperitoneally with a dose of 200 $TCID_{50}$ of virus, most were aborted and the 8 which were born normally died within 5 days.

However, one of 13 new born hamsters injected with 200 $TCID_{50}$ of virus by the intrapleural route developed subcutaneous tumors within 45 days after delivery. Furthermore, 2 of 5 new born animals which received a dose of 400 $TCID_{50}$ of the virus developed

TABLE 1. *Rate of tumor induction in hamsters on injection of various doses of Adenovirus type 12 by different routes into fetuses*

Virus titer $TCID_{50}$/ml	Inj. dose ml	Route of injection into fetus	Days before delivery of injection	No. of fetuses tested	No. of new born	Days before sacrifice	Rate of tumor development
None[a]	0.02	Intrapleural	2–4	17	8	150	0/ 4
10^3	0.01	Unidentifiable[b]	8–9	16	13	102	0/12
10^4	0.04	Unidentifiable[b]	7	7	2	84	0/ 2
10^3	0.02	Amniotic cavity	4–5	32	15	104–107	0/12
10^4	0.02	Intracranial	2–4	31	Aborted		
10^4	0.02	Intrapleural	2–5	49	17	148–151	1/13
10^4	0.04	Intrapleural	3	20	5	43– 70	2/ 5
10^4	0.02	Intraperitoneal	2–4	20	8	Died within 5 days	

a: Tissue culture fluid.
b: Fetuses were not large enough to permit exact identification of the injection site.

subcutaneous tumors within 50 days, but no tumors developed in the intrapleural or intra-abdominal regions. Further study is necessary to clarify the observation (Table 1) that inoculation of fetuses was less sensitive than that of new born rats for detection of oncogenic activity. Histologically, the subcutaneous tumors seemed to be immature neurognic neoplasma, as previously reported by various workers (Ogawa et. al., 1966, Yoshida et. al., 1966).

Studies on the development of tumors in hamsters by placental transfer were made by intravenous injection of 5×10^4 or 4×10^4 $TCID_{50}$ of virus into mothers. Forty one fetuses of mothers injected by this route, were born normally and did not developed any tumors within 9 months after birth.

Furthermore, 51 fetuses were removed 30, 60 and 180 min after intravenous injection of virus into 6 pregnant hamsters. The fetuses were minced, frozen and thawed. Isolation of virus from this suspension employing HeLa cells was not successful.

REFERENCES

Bellanti, J. A., M. S. Artenstein, L. C. Olson, E. L. Buescher, C. E. Luhrs, and K. L. Milstead. 1965. Congenital Rubella. Amer. J. Dis. Child. 110: 464–472.

Galton, M. and L. Kilham. 1966. Chromosomes of "Mongoloid" hamsters. Proc. Soc. Exp. Biol. Med. 122: 18–22.

Kono, R., T. Tsuchizaki, S. Nasu, S. Kujima, K. Fujiwara, T. Kumagai, M. Matsumoto, K. Yamakawa and Y. Ikawa. 1968. Symposium on vertical transmission of viruses in Tokyo. To be published in Virus 18: 143–184 (in Japanese).

Ogawa, K., A. Tsutsumi, K. Iwata, Y. Fujii, M. Ohmori, K. Taguchi and Y. Yabe. 1966. Histogenesis of malignant neoplasm induced by adenovirus type 12. Gann 57: 43–52.

Toolan, H. W. 1960. Experimental production of mongoloid hamsters. Science 131: 1446–1448.

Trentin, J. J., Y. Yabe and G. Taylor. 1962. The quest for human cancer viruses. Science 137: 835–841.

Yoshida, N., K. Fukui, K. Sumitomo, S. Kimura and S. Yamashita. 1966. Studies on oncogenic adnoviruses. 1. Confirmation of carcinogenicity of adenovirus type 12 for new born hamsters. Shikoku Acata Medica 22: 83–85 (in Japanese).