Journal of Oral Pathology 1984: **13**, 686–691

Salivary gland tumors induced by ^{32}P

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The oncogenic power of ^{32}P was demonstrated in salivary glands. An intraglandular injection of 0.25 mCi of chromic colloidal phosphate (^{32}P) was administered to young adult Wistar rats. Seven months post-injection, tumors began to appear in the neck region in 64% of the rats. The tumors were sarcomas (50%), carcinomas (35.70%), and carcino-sarcomas (14.28%).

Accepted for publication March 26, 1984

Tumor induction in salivary glands of mice, rats and hamsters has been obtained mainly with chemical carcinogens, either in the form of pellets implanted within the gland (1–6), or in the form of solutions injected into the gland (7–8). Other experiments in tumoral induction in salivary glands were done with the SE polyoma virus (9–10). Law and Dawe (11) did this on animals previously irradiated by X rays to provoke inmunodepression, while Cunningham et al. (12) produced experimental tumors with cytomegalovirus. Others have analyzed the serial transplantations of tumors induced by different chemical carcinogens such as DMBA (13–15).

There is literature on the carcinogenic power of irradiation in human salivary glands, referring either to survivors of Hiroshima and Nagasaki (16–17) in whom a greater tumoral incidence has been demonstrated, or to patients in whom tumors have appeared after local irradiation treatments (18–21).

However, there is little information about experimental tumor induction by irradiation of the salivary glands (22–24). We have found no data on induction of tumors in salivary glands with radioactive isotopes. In our laboratory, the great oncogenic power of ^{32}P has been demonstrated in joints and subcutaneous tissue (25). When ^{32}P is injected in the form of colloidal chromic phosphate, it acts as an internal source of irradiation until decay. This experiment was designed to prove the inductive power of a radioactive colloidal solution of ^{32}P in salivary glands of rats.

Material and methods

Fifty-two male Wistar rats weighing 150 g at the beginning of the experiment were used. Twenty-six received an injection of 0.005 ml of radioactive colloidal chromic phosphate (^{32}P) (activity 0.25 mCi, particles: 10 to 30 nm) (26) into the left submaxillary gland (SBM). Another 26 were injected in the same way with cold colloidal chromic phosphate. In all

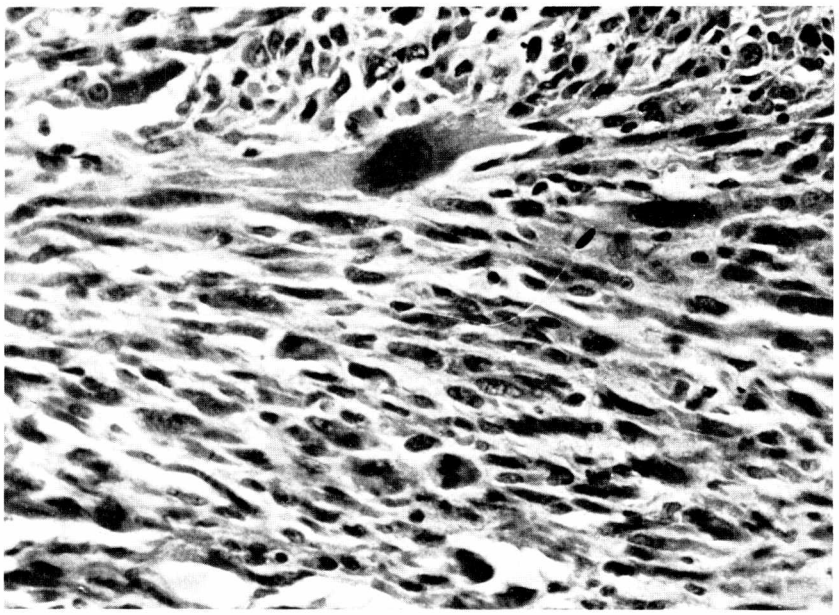

Fig. 1. Characteristic features of the fibrosarcomas with a herring-bone pattern, atypical undifferentiated cells and a large number of mitotic figures (× 240).

cases the glands were surgically exposed before injection.

Four animals of the control group were killed 2 weeks after injection and the glands processed for light microscopy. When an animal developed an ulcerative or nodular lesion, it was killed with ether. Each animal, was autopsied in search of metastases. At the end of the experiment, an equivalent number of animals injected with cold colloidal solution was killed.

In both groups, glands were processed for light microscopy.

Results

In the early days following the injection, most of the animals showed an epilation around the gland region. Four animals injected with the radioactive solution died within 30 days of injection, due to causes unconnected with the experiment.

The glands of animals killed 2 weeks after injection were seen to contain the colloidal solution under light microscopy.

Nodular and ulcerative tumors started to appear (1 case) from Day 240, 4 more appeared between Days 240 and 270, 4 more between 270 and 330, another 3 on subsequent days up to 480, and , finally, 2 more appeared between Days 480 and 560. In all, 14 tumors were detected, an incidence of 63.63%.

Their aspect varied from nodular formations (10 cases) to ulcerated areas with raised edges (4 cases). The lesions were in the neck, over the SBM gland region. In one case, the tumor appeared below the gland region, over the joint of the sternum with the clavicle. One

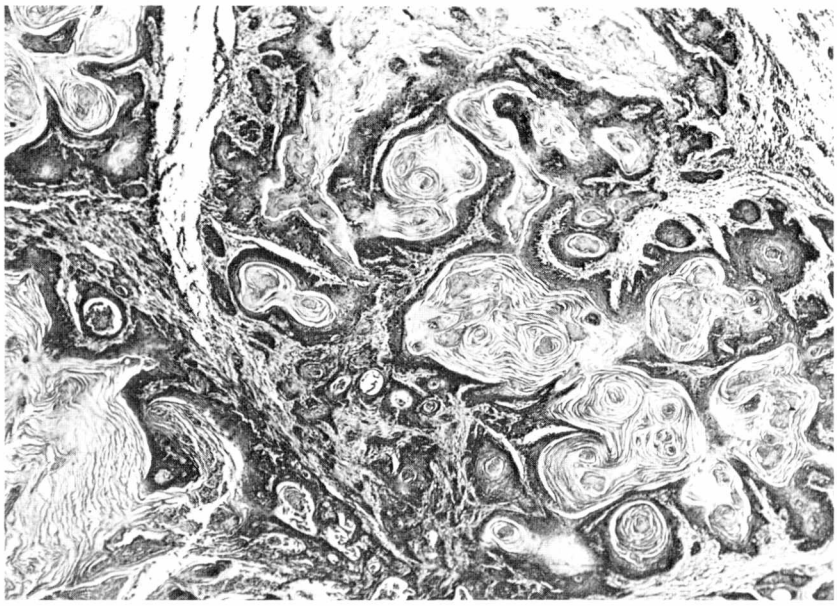

Fig. 2. Squamous cell carcinoma developed in an injected gland showing abundant horn-pearl formation in cystic spaces (× 90).

animal with a large tumor died spontaneously. The size of the tumors varied, the largest measuring 6 × 3 × 3.5 cm.

Microscopic observations of the tumors showed 2 different histological types of sarcomas and carcinomas, and in 2 cases, the tumors were carcinosarcomas. Of all the tumors detected, 50% were sarcomas. These tumors were highly cellular, with predominant spindle-shaped cells arranged in bands forming a herring-bone pattern in some areas.

There were numerous vascular spaces, often in the form of clefts, lined by a monolayer of endothelial cells, and with hematic intraluminal content. The sarcomas showed collagen formation and spindle-shaped and pleomorphic cells. Some were large with hyperchromatic atypical nuclei, others had double nuclei. The mitotic index was high and isolated necrotic foci were observed. All the tumors were infiltrative (Fig. 1).

In 35.7%, the microscopic features were of squamous cell carcinomas. In 2 cases, carcinomas originating in the skin invaded the gland. In other cases, the glandular origin of the tumor was clearly detectable. Horn-pearl formations, high mitotic index, cystic spaces and infiltrative activity were always observed (Fig. 2).

In 2 cases (14.28%) a mixture of carcinomatous and sarcomatous areas were found and these were diagnosed as carcinosarcomas.

In other cases there were some glandular patterns with ductal formations (Fig. 3).

In tumors originating in the skin, some areas of a glandular parenchyma showed atypical structures, binucleate cells, hyperchromatic nuclei and a considerable number of mitotic figures. The autopsies did not reveal macroscopic metastases.

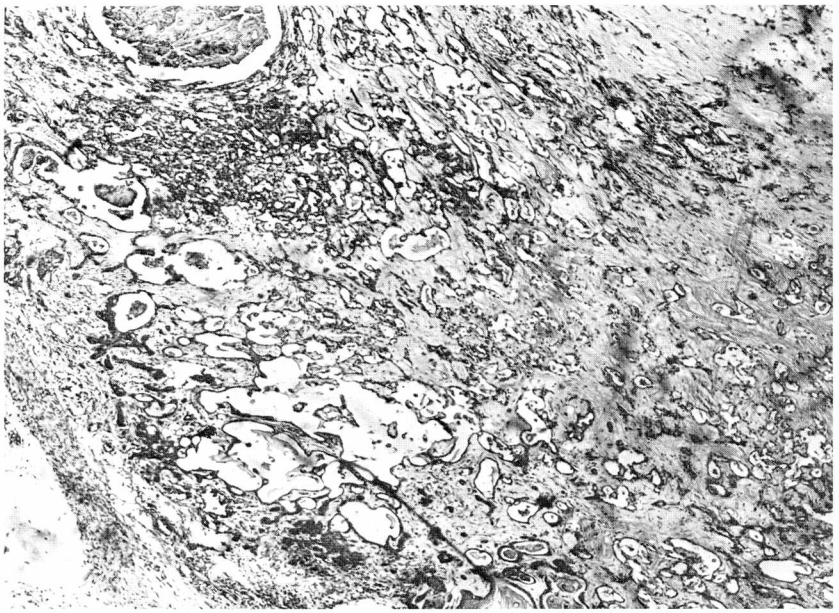

Fig. 3. Glandular pattern in a squamous cell carcinoma displaying ductal formations (× 60).

Discussion

The oncogenic power of ^{32}P was demonstrated in salivary glands, and had been demonstrated before in joints and subcutaneous tissues (25). As no histological alterations were found in the salivary glands injected with the cold colloid, the radioactive isotope is demonstrated to be responsible for the tumoral induction.

The minimal latency period in this experiment was similar to that found in other tissues with the same radioactive substance (25), or with other forms of irradiation (22–24), but was longer than that observed in glandular tumors induced by chemical carcinogens (1, 15, 27, 28, 29, *inter alia*).

As for the histological type of tumors induced in the gland by the radioactive colloid, 50% were sarcomas and 35.70% carcinomas. Other authors have similarly reported induction of tumors of different origins by chemical carcinogens (induced carcinomas (1), and 3, 6, 27, fibrosarcomas). Shafer (4), among many other authors, obtained carcinomas as well as sarcomas. Most authors agree in explaining the origin of epithelial tumors in ductal metaplasias which could produce cystic cavities with keratin content. It was a striking feature to see the development of 2 carcinosarcomas (14.28%). Experimental carcinosarcomas rarely develop.

The histological origin of the tumors induced by the radioactive colloid may have varied because the cells of the target organ, i.e., epithelial cells or fibroblasts and other cells of the stroma, were different. The tumors at the edges of the skin ulcers might be explained as a peripheral localization of the radioactive colloid in the gland with a carcinogenic effect on the epidermis. Similar results were obtained by Ubios et al. (25) with knee injections.

In this series of experiments all tumors induced were malignant. However, other authors have reported benign tumors. The induction of adenomas in SBM glands was obtained by Cherry and Glucksman (23) and Glucksman and Cherry (24), from 7 months post-irradiation Takeichi et al. (22) induced glandular tumors with X irradiation applied locally in a single dose of 2800 rads and in multiple doses of 500 rads, with total doses of 3000 and 5000 rads. He evaluated hormonal participation by performing ovarectomies. The tumors were adenomas, atypical proliferations, and cancer. Law and Dawe (11) also obtained pleomorphic adenomas with SE polyoma virus and irradiation.

Colloidal solutions remain at the injection site without spreading over great distances, and thus, the isotope acts as an internal source of radiation until total decay, generating an active irradiation zone that provides a dose of maximal oncogenic activity at the site. This may explain the high incidence of tumors found in this experimental model, and, thus, constitutes an appropiate method for the study of tumor induction by irradiation.

Acknowledgements

This paper was partly supported by grant N° 10676/83-13 from the SUBCYT. The authors wish to thank Miss Ofelia Chiesa and Miss Patricia Mandalunis for their technical assistance.

References

1. Steiner PE. Comparative pathology of induced tumors of salivary glands. Arch Path 1942: 34: 613.
2. Bauer WH, Byrne JJ. Induced tumors of the parotid gland. Cancer Res 1950: 10: 755.
3. Cataldo E, Shklar C. Chemical carcinogenesis in the hamster submaxillary gland. J Dent Res 1964: 43: 568.
4. Shafer WG. Experimental salivary gland tumorigenesis. J Dent Res 1962: 41: 117–124.
5. Cataldo E, Shklar G, Chauncey A. Experimental salivary gland tumors in rats. Arch Pathol 1964: 77: 305.
6. Chauncey A, Shklar G, Quintarelli G. Histochemistry of experimentally induced fibrosarcoma in rat submaxillary gland. J Dent Res 1961: 40: 684 (abstr).
7. Berberich J. Experimentelle Erzengung von Tumoren am Ohr, an der Nase, den Nabenhohlen und der Parotis. Z-Hals-Nasen u Ohrenh 1932: 31: 343.
8. Chaudry AP, Reynolds DH, Gorlin RJ, Vickers RA. Experimental carcinogenesis in submandibular glands of hamsters. J Dent Res 1961: 40: 426.
9. Stewart SE. Neoplasms in mice inoculated with cell-free extracts or filtrates of leukaemic mouse tissues. I: Neoplasms of the parotid and adrenal glands. J Nat Cancer Inst 1955: 15: 1395.
10. Fleming HS. Polyoma-virus and the teeth. J Dent Res 1963: 42: 1405.
11. Law LW, Dawe CJ. Influence of total body X irradiation on tumor induction by parotid tumor agent in adult mice. Proc Exp Biol Med 1960: 105: 414.
12. Cunningham BD, Sinus RA, Zimmerman ER, Byrd DL. Murine tumor induction by cytomegalovirus. Oral Surg 1975: 40: 130.
13. Cappucino C, Shklar G. A transplantable salivary gland tumor model in rat. Arch Oral Biol 1976: 21: 147.
14. El Asfahani A, Higashi GI, Ahmed MA. Chemical carcinogenesis of submandibular gland in BALB/c mice and syngeneic passage of the tumor. Oral Surg 1979: 48: 47.
15. Cataldo E, Reif AE. An explanation of the proportion of carcinomas and sarcomas seen in chemically induced murine submaxillary gland tumors. Cancer 1982: 50: 531.
16. Belsky JL, Tachikawa K, Cihak RW, Yamamoto T. Salivary gland tumors in atomic bomb survivors, Hiroshima-Nagasaki 1957–1970. JAMA 1972: 219: 864.
17. Takeichi N, Hirose F, Yamamoto H. Salivary gland tumors in atomic bomb survivors, Hiroshima-Japan. I: Epidemiologic observations. Cancer 1976: 38: 2462.

18. Son JH. Radiation carcinogenesis. Cancer 1975: 36: 941.
19. Modan B, Mart H, Baidatz D, Steinitz R, Levin SG. Radiation-induced head and neck tumors. Lancet 1975: 1: 277.
20. Hazen RW, Pifer JW, Tozooha ET, Livingood J, Hempelmann LH. Neoplasms following irradiation of the head. Cancer Res 1966: 26: 305.
21. Swelstad JA, Scanlon EF, Iviedo MA, Hugo NE. Irradiation induced polyglandular neoplasia of the head and neck. Am J Surg 1978: 135: 820.
22. Takeichi N, Hirose F, Tikizawa S, Watanabe H, Ootsuka S. Salivary gland tumors in rats following local X irradiation. Nagasaki Igakkai Zasshi 1976: 51: 196.
23. Cherry CP, Glucksman A. Injury and repair following irradiation of salivary glands in male rats. Br J Radiol 1959: 32: 596.
24. Glucksman A, Cherry CP. The induction of adenomas by the irradiation of salivary glands of rats. Radiat Res 1962: 17: 186.
25. Ubios AM, Silberman FS, Cabrini RL. Radioactive – induced tumors by phosphorus 32 as colloidal compound. Cancer 1983: 51: 1605.
26. Anghileri LJ, Marques R. New colloidal chromic radiophosphate (^{32}P) for local irradiation of the central nervous system. Int J Appl Radiat Isotope 1967: 18: 97.
27. Brown AM. A comparative assay of the *in vitro* and *in vivo* behavior of three chemically induced neoplasms of rat submandibular glands. J Oral Path 1973: 2: 33.
28. Standish SM. Early histologic changes in induced tumors of the submaxillary salivary glands of the rat. Am J Path 1957: 33: 671
29. Fonseca MM, Rins de David ML, Gendelman H. Estres y Patología experimental inducida con DMBA en glándulas salivares de la rata. Rev Fac de Odont de la Univ Nac de Córdoba 1981: 13: 77.

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