Fibrosarcoma induced by administration of methyl cholanthrene into post extraction sockets of mandibular molars in rats

J. Sela and D. Harary

Division of Oral Pathology, Hebrew University-Hadassah, School of Dental Medicine, P.O. Box 1172, Jerusalem (Israel),
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Summary. Methyl cholanthrene in crystalline form and in a slow releasing vehicle was introduced into mandibular post extraction sockets. Fibrosarcomas developed within 9 months.

Experimental induction of neoplasms in oral and other tissues of laboratory animals has attracted extensive interest. Methyl Cholanthrene (MCA) has been used for this purpose[1-3]. In previous experiments we could demonstrate induction of fibrosarcomas by administration of DMBA (9, 10-diamethyl-1,2-benzanthracene) using different methods in the parotid glands and alveolar bone in rats[4,5]. Administration of DMBA into alveolar bone sockets failed to induce osteosarcomas. It was suggested that nondevelopment of osteosarcomas in our experimental setup could be attributed to the use of DMBA. Other investigators were successful in inducing osteosarcomas using MCA[1]. The purpose of the present work was to study the direct effect of MCA, in the crystalline form and suspended in a slow releasing vehicle, on the tissues involved in the healing process of alveolar bone sockets in rats.

55 albino rats of either sex of the Hebrew University (Sabra) strain weighing 100 g each were used. The animals were anesthetized and the mandibular 1st and 2nd right molars were extracted. Pellets of 0.5 ml vitepsol H-15 (Dynamit Nonel AG, 5000 Köln-Mülheim 1, FRG) containing 10% MCA (20-methyl cholanthrene, Sigma Chemical Co. St. Louis MO 63178 USA) were introduced into the sockets of 29 rats, sutures were placed in order to prevent the removal of the carcinogen; of this group 14 rats received 3 additional injections of 0.5 cm^3 emulsified pellets of 10% MCA in vitepsol H-15 (at 35 °C). These were introduced into the operation site 10, 20 and 30 days after the extraction of the molars. The remaining 15 rats of the 1st group received a single injection of 0.5 ml of emulsified MCA in vitepsol H-15 into the operation site 20 days after the extraction. The 2nd group consisted of 26 rats that received crystalline MCA into the sockets after the extraction. This group was divided into 2 subgroups of 15 and 11 rats. The 15 rats of the 1st subgroup received a single injection of 0.5 ml of emulsified MCA in vitepsol H-15 after 20 days. 3 similar injections were introduced into the operation site of the remaining 11 rats 10, 20 and 30 days after the operation. The animals were kept for 9 months under surveillance and roentgenographed periodically; 6, 9, 6 and 7 rats survived out of 14, 15, 15 and 11 rats of the 4 subgroups respectively, the rats were killed and their heads were fixed in formalin. Paraffin embedded sections were cut at 6 µm and stained with haematoxylin-eosin, Masson's Trichrome method for connective tissue and Periodic Acid Schiff for polysaccharids. All the surviving rats developed neoplasms, the diameters of these swellings ranged from 5 to 8 cm. The lesions derived from the sockets and invaded the adjacent tissues. Histopathological examination revealed that the tumors were fibrosarcomas (figure 1). This observation was supported by the positive staining of fibriles with Masson's Trichrome method for connective tissue (figure 2).

Production of fibrosarcoma by administration of MCA into the sockets of rat mandibles was surprising in view of results published by Levy et al.[1], who in a similar experimental setup obtained osteosarcomas. In the present study, we used crystalline MCA in 1 group in order to repeat the experimental conditions of the previous researchers[1]. We could neither induce osteosarcoma by this method nor could we do so in an additional group of rats in which MCA was implanted in pellets of a slow releasing vehicle. This experimental group was designed in view of the study of Yamamura et al.[2] who have pointed out the importance of the continuous action of carcinogens. In our case, the MCA was embedded in the mandibular socket immediately post exodontia and was operative during the healing process which involved formation of bone matrix, ossification, resorption, production of mesenchymal, epithelial and other tissues. Non induction of osteosarcomas by the method described was identical to the results of our previous experiments. Nevertheless, the constant production of fibrosarcomas in all these experiments makes the method highly recommendable for this purpose.

Implantation of MCA in artificial defects drilled in the incisor root area of mice induced osteosarcoma[1]. The

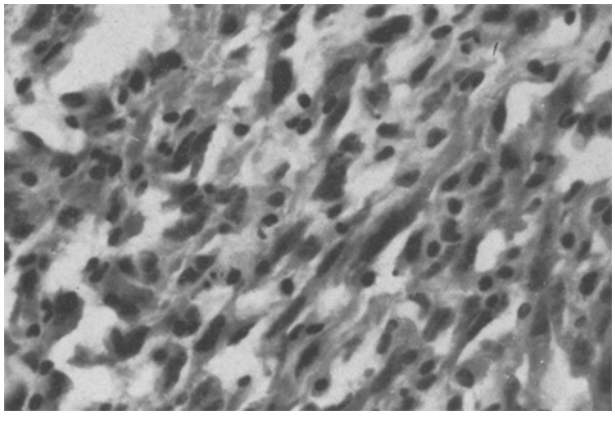

Fig. 1. Fibrosarcoma, hyperchromasia, atypism and pleomorphism of cells and nuclei. Haematoxylin and Eosin. × 40.

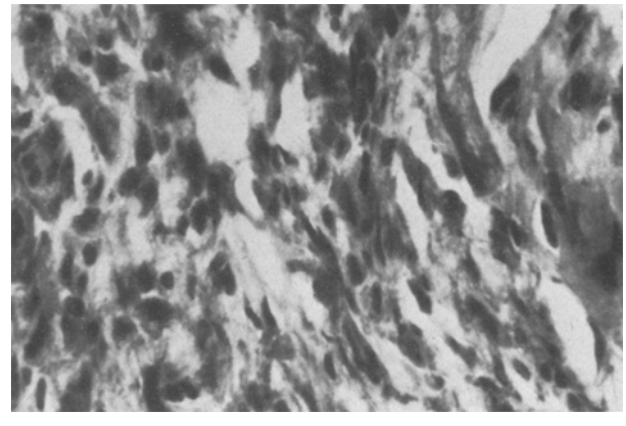

Fig. 2. Formation of bundles of collagen fibriles. Masson's Trichrome. × 40.

diversity of these previous results, as compared to the present study, may be due to the differences in animal species and in the exact loci of implantation of the carcinogen. It is of interest to note that the duration of the experiment, i.e. 9 months, was identical in the different studies. The convenience of production of neoplasms such as osteosarcoma by the administration of carcinogens intrigued us to study this experimental model. However, further evaluation of this method aiming at the induction of osteosarcoma is required.

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The effect of X-irradiation on the amount of dopamine in corpus striatum of the rat

S.R. Stepanović and Jelena V. Nikolić

Boris Kidrič Institute, P.O. Box 522, YU-11000 Beograd (Yougoslavia), 3 May 1978

Summary. The effect of ionizing radiation on the amount of dopamine in corpus striatum was investigated in rats exposed to 650 or 850 R of X-rays. The amount of dopamine in the corpus striatum was measured fluorimetrically in various periods of time after irradiation. It was found that, irrespective of the dose applied, the ionizing radiation caused a significant depletion of dopamine in the striatum.

The dopamine was first detected by Goodall[1,2] in the heart and in the adrenal medulla. The presence of this catecholamine was later demonstrated in a variety of organs and in the central nervous system[3-6], the highest concentration of dopamine being found in the corpus striatum[6-8]. Ionizing radiation has been known to affect catecholamine (noradrenaline, adrenaline) stores in the adrenal medulla, heart and brain[9-14]. However, until now no data have been available concerning the changes of dopamine content in the brain of irradiation animals. It was therefore decided to study the effect of irradiation on the amount of dopamine in its typical store, the corpus striatum.

Materials and methods. Male rats weighing 190–210 g were used. The animals were whole-body X-irradiated with 650 or 850 R. Irradiation parameters were: 200 kV; 0.5 mm Cu; D-42 cm. The dose rate was 112 R/min. The irradiated animals were sacrificed 24 and 48 h, 5, 7 and 14 days after irradiation. Each experimental group had its day-to-day controls. The brain was rapidly removed and the striatum of both sides was dissected on an ice-cold beaker. The mean weight of the striatum was about 90 mg. The striata from 2 rats were pooled. Method of Manuhin et al.[15], based on the methods of Carlsson and Waldeck[16] and Laverty and Taylor[17], was used for extraction and quantitative estimation of dopamine. Recoveries of dopamine were 80–90% throughout the experiment. Fluorimetric estimation was done on an Aminco-Bowman spectrophoto-fluorimeter.

Results and discussion. The present experiments show that the amount of dopamine in the corpus striatum of rats irradiated with 650 R was significantly decreased 24 h to 7 days following irradiation, as compared with the control values. However, dopamine stores were completely restored 14 days after irradiation. The amount of dopamine in corpus striatum of irradiated animals was the same as non-irradiated controls. The results are presented in table 1. In rats irradiated with the dose of 850 R, the amount of dopamine in the striatum was also significantly decreased at all time intervals from 24 h to 7 days after irradiation (table 2). In these animals, there was no replenishment of normal dopamin stores during the observed period of time. The normal noradrenaline content in the corpus striatum of rats is small and amounts only to few percent of the total catecholamine content. Therefore, the presence of noradrenaline in corpus striatum could not be detected, using

Table 1. The amount of dopamine in the corpus striatum of the rat irradiated with 650 R at different time intervals after irradiation (mean ± SE µg/g fresh tissue). The number of experiments is indicated in parenthesis

Controls	Period after irradiation				
	24 h	48 h	5 days	7 days	14 days
1	*2*	*3*	*4*	*5*	*6*
8.58±0.23 (25)	5.21±0.28 (14)	4.80±0.37 (14)	5.57±0.26 (18)	5.86±0.19 (18)	8.39±0.46 (15)

p *(1:2)* < 0.001; p *(1:3)* < 0.001, p *(1:4)* < 0.001, p *(1:5)* < 0.001.

Table 2. The amount of dopamine in the corpus striatum of the rat irradiated with 850 R at different time intervals after irradiation (mean ± SE µg/g fresh tissue). The number of experiments is indicated in parenthesis

Controls	Period after irradiation			
	24 h	48 h	5 days	7 days
1	*2*	*3*	*4*	*5*
8.50±0.31 (20)	5.18±0.35 (15)	5.27±0.26 (15)	5.00±0.21 (19)	4.12±0.33 (11)

p *(1:2)* < 0.001; p *(1:3)* < 0.001; p *(1:4)* < 0.001; p *(1:5)* < 0.001.