COMMUNICATION

[Neovascularization During Hamster Cheek Pouch Carcinogenesis Induced by 7,12-Dimethylbenz[α]anthracene*]

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The hamster cheek pouch is one of the more versatile organs for experimental studies on chemical carcinogenesis, tumor transplantation, or vascular changes. Substantial functional vascular changes were observed in hamster cheek pouch epithelium bearing tumors induced by repeated topical applications of 7,12-dimethylbenz[α]anthracene (DMBA). However, it is not clear whether these hemodynamic changes are due to neovascularization or not. We examined the relationship of preneoplastic and neoplastic epithelial changes to anatomic vascular changes in the hamster cheek pouch during and after DMBA carcinogenesis.

Check pouch of 100 male golden hamsters was treated with 0.05 ml of a 0.5% solution of DMBA in mineral oil 3 times a week for 14 consecutive weeks, with no application during weeks 3, 7, and 11. The carcinogen solution was applied with a needleless tuberculin syringe into the right pouch. Changes in the cheek pouch were examined by scanning electron microscopy of vascular casts and by histopathological examination after sacrifice at 2, 4, 6, 8, 10, 12, 14, 20, and 30 weeks after the start of DMBA treatment.

Hamsters were anesthetized with intraperitoneal injection of pentobarbital sodium and the right cheek pouch was inflated with 2–3 ml of warmed 0.9% NaCl. A cannula was inserted into the left ventricle of the heart, then through the brachiocephalic artery into the right common carotid artery, and ascending and descending aortae were commonly ligated. The cheek pouch vasculature was perfused with warmed 0.9% NaCl, drainage being from small incision in the left and right subclavian veins at the subclavicular fossa. Once the perfusate was clear, acryl modified polyester resin, Mercox CL-2B (Dainippon Ink and Chemicals Inc., Tokyo), was injected through the cannula. The right cheek pouch was then ligated at its neck, excised in toto, and digested with 25% NaOH. This left only the polymerized vascular casts, which were observed with a scanning electron microscope (Hitachi HHS-2R).

Normal cheek pouches had loose plexi of capillaries beneath the epithelia. The capillaries were relatively uniform in diameter, present in low density, and appeared in horizontal wave-like patterns, especially near the neck of the pouches (Photo 1, × 40). They were connected to larger vessels in the deeper layers of the cheek pouch. Small foci of high-density plexi of small diameter capillaries with multiple short terminal branches, type I, were occasionally seen in cheek pouches treated with DMBA (Photo 2, × 180). Type I foci were greatest in number at week 4 and subsequently diminished or disappeared by week 14. Type III foci,
consisting of highly tortuous capillary loops without terminal branches, were first found after 6 weeks. The number and extent of type III foci increased gradually and corresponded with the extent of hyperplastic, papillomatous, and carcinomatous changes. The diameter of capillaries in type III foci was generally larger than that of normal capillaries and was fairly uniform through week 10. After 14 weeks, however, the capillaries became irregular from area to area and their diameter was considerably larger than that of normal capillaries. These variations in type III foci were most pronounced in carcinomas (Photo 3, ×50).

Histopathologically, erosion and shallow ulceration were initially found. These changes peaked at week 4 and substantially diminished or disappeared after week 14. Mild papillary or nodular hyperplasia was found after week 6, and small papillomas were found after week 10. Squamous cell carcinomas were found after 14 weeks.

Types I and III vascular foci were similar to those observed in rat bladder carcinogenesis by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN),\textsuperscript{4,5} but type II foci were not found during cheek pouch carcinogenesis. Type I foci may represent repair of direct damage to blood vessels by inflammation and necrosis or by direct toxic action of DMBA on the endothelium. Type III foci showed close relationship to epithelial proliferative lesions, papillary or nodular hyperplasia, papillomas, or carcinomas, and appear to represent endothelial responses to secretion of an angiogenesis factor from malignant or possibly premalignant tissues.\textsuperscript{1,5}

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REFERENCES