INDUCTION OF MALIGNANT TUMORS IN MICE BY METHYLCHOLANTHRENE BOUND COVALENTLY WITH BOVINE SERUM ALBUMIN

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UDC 616-006.04-092.9

The carcinogenic properties of 3-methylcholanthrene, covalently (by means of bis-diazotized benzidine) bound to protein, were studied in experiments on mice. It was shown that binding with protein in this way does not abolish carcinogenic activity: Sarcomas developed rapidly at the sites of subcutaneous injection of the conjugate. For this reason, immunization of animals with conjugated 3-methylcholanthrene-protein antigen in order to obtain antibodies specifically binding the carcinogen is difficult.

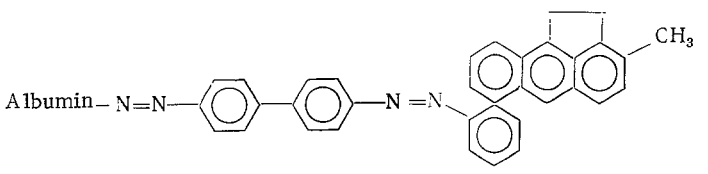
KEY WORDS: carcinogen; carcinogen-protein conjugate; antibodies.

Extensive information has now been obtained to show that different toxic chemical agents can be neutralized *in vivo* by specific antibodies [1, 3]. Attempts have been made [4-6] to neutralize some carcinogens by preliminary immunization of animals with conjugated antigen, containing molecules of the carcinogen as hapten and a protein as the carrier. As long ago as in 1939 it was reported [5] that the development of a tumor induced by 1,2,5,6-dibenzanthracene is inhibited after immunization of mice by a conjugate of this carcinogen with protein. It was later shown that injection of free 2-anthrylamine into immunized animals possessing antibodies against 2-anthrylamine has a weaker carcinogenic action than in nonimmunized animals [6]. However, the possibility of immunologic neutralization of various carcinogenic substances is not yet completely clear. Moreover, the writers have shown that covalent binding of certain pharmacological agents with proteins does not lead to the loss of their biological activity *in vivo*, but, because of changes in their pharmacokinetics as the result of the macromolecular carrier, it is manifested chiefly at the level of endocytic cells [2].

In the investigation described below an attempt was made to study whether it is possible to protect an animal against the carcinogenic effect of 3-methylcholanthrene (MCh) by immunization with a conjugated antigen consisting of MCh and bovine serum albumin (MCh-BSA). At the same time, the presence or absence of carcinogenic properties in the macromolecular compound to be tested was studied.

EXPERIMENTAL METHOD

The MCh-BSA conjugate was obtained with the aid of bis-diazotized benzidine:



A solution of 4 g BSA in water was added to a solution of 700 mg MCh in dioxan in a volume

Department of Immunopharmacology, Research Institute for Biological Testing of Chemical Compounds, and Department of Carcinogenic Agents, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 3, pp. 332-333, March, 1980. Original article submitted February 8, 1979.

 0007-4888/80/8903-0338$07.50

so that the ratio of dioxan to water did not exceed 1:1. A freshly prepared solution of bis-diazotized benzidine, obtained by interaction between 700 mg benzidine and 525 mg sodium nitrite in acetic acid was added slowly to the resulting solution, during cooling to 0°C. The reaction mixture was kept for 2 h and dialyzed, first against a solution of dioxan in water (1:1), and later against distilled water. The conjugated MCh-BSA antigen, which we synthesized, was tested in line A mice (body weight 18-20 g at the beginning of the experiment). Observations were made on four groups of mice, with 25 females and 17 males in each group. Group 1 consisted of mice receiving 5 (once a week) subcutaneous injections, each of 0.5 ml of an aqueous suspension of the MCh-BSA conjugate in a dose of 2.6 mg per mouse into the interscapular region; group 2 consisted of mice receiving the conjugate by the same scheme and, at the same time, receiving an oily solution of MCh (0.3 mg in 0.3 ml sunflower oil) in four daily doses per os; group 3 consisted of mice receiving MCh only, per os (by the same scheme as in group 2); group 4 (control) consisted of intact mice.

EXPERIMENTAL RESULTS

Small firm nodules were found in the mice of groups 1 and 2, 6-8 weeks after the end of the experiment at the site of injection of the conjugate. The nodules attained a considerable size after 10-12 weeks, and after 14-16 weeks most of the mice had died; the rest were killed and their condition was poor. Among the animals of group 1, at the site of injection of the MCh-BSA conjugate, tumors were found in 94% of males and in 76% of females ($P>0.1$). Among the mice of group 2, subcutaneous tumors were found in 94% of males and in 96% of females (the difference was not significant compared with data for group 1, $P>0.1$). Histological investigation of the tumors showed that they were fibroblastic sarcomas. No other neoplasms were found in the experimental mice of groups 1-3 and in the control mice killed at the same time.

The experiments thus showed that MCh, bound covalently with protein, does not lose its carcinogenic properties. The ability of another conjugate (1,2,5,6-dibenzanthracene-carbaminocasein), incidentally, was described previously [5]. Rapid development of subcutaneous sarcomas arising after injection of MCh-BSA antigen, and causing death of the animals, did not permit an investigation of the effect of immunization by this antigen on the carcinogenic effect of free MCh. However, the results may evidently be of some theoretical interest. The fact is that conjugates of carcinogens, notably MCh, with protein may be formed *in vivo* under the influence of microsomal oxidases with mixed function. For many substances microsomal oxidation, supplemented by binding with albumin, which induces antibody formation, represents the optimal detoxicating system [1]. Whether the mechanism of covalent binding of polycyclic hydrocarbons with the body proteins plays a role in detoxication requires further investigation.

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