DIFFERENCE IN THE INDUCTION OF OSTEOSARCOMA IN RABBIT BONE WITH SINGLE ADMINISTRATION OF THREE KINDS OF CHEMICAL CARCINOGENS

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A single intramedullary administration of each dose (15~20 mg) of 4-nitroquinoline 1-oxide, 3-methylcholanthrene, or 7,12-dimethylbenz[a]anthracene was applied to the mandible, diaphysis, or distal metaphysis of the femur of rabbits. The highest incidence in production of osteosarcoma was obtained from the group in which 4-nitroquinoline 1-oxide was applied to the distal metaphysis (75%, including one case of chondrosarcoma). Tumors hardly appeared in any of the groups when given 3-methylcholanthrene or 7,12-dimethylbenz[a]anthracene. Histologically, three kinds of entities were recognized from the quantitative difference of the reactive tissues which appeared around carcinogens. It is estimated that the condition of entity III induces the highest incidence of osteosarcoma if chemical carcinogens are given into the bone marrow of experimental animals.

Melnikov and Kondratiena,5) Yamada,8) Stanton,7) and Sato and Horikoshi6) reported the production of experimental osteosarcoma by using chemical carcinogens. So far, it is difficult to obtain osteosarcoma in bones compared to malignant tumors in other organs. This difficulty comes from dose of the chemicals applied, method and topographical site of the body of administration, and species of animals. In the present work, the factors mentioned above were investigated with three kinds of carcinogens applied to anatomically and physiologically different parts of a skeleton of rabbits. Osteosarcoma means malignant bone-forming sarcoma and is used synonymously with osteogenic sarcoma described in our earlier report.6)

Materials and Methods

Animals: Both sexes of mixed breed white rabbits, weighing 2~3 kg, were used.

Chemical carcinogens: 4-Nitroquinoline 1-oxide (4-NQO), 3-methylcholanthrene (3-MC), and 7,12-dimethylbenz[a]anthracene (DMBA) were used. Each agent was resolidified before the experiments by the method described earlier.6) Treatment: Each carcinogen was applied once into the medullary cavity of the mandible, middle part of the diaphysis, or distal metaphysis of the femur using the same method as reported previously.6) Careful attention was paid to leakage of the chemicals from the cavity and also to the injury of the joint capsule at the distal metaphysis. After 6 months, all of the animals were sacrificed for autopsy and roentgenography. Histological specimens were prepared as reported earlier.6)

Results

Histological examination of induced tumors revealed a number of osteosarcomas, a few fibrosarcomas, chondrosarcomas, and cementoblastic fibromas (Table I). These tumors showed a striking histological similarity to those observed in human tumors. Metastasis of osteosarcoma to the lung was
observed in one case. From the histological results, the most effective condition in inducing osteosarcoma was observed in the group, in which 4-NQO was applied in the medullary cavity of the distal metaphysis of the femur (with the highest incidence of 75%, including one case of chondrosarcoma) (Photos 1, 2, and 3). In the group in which 4-NQO was applied to the mandible, carcinogenicity showed nearly the same rate (60%) as that in the former report.4) Thus, 4-NQO in these two sites exhibited a distinguished carcinogenicity for induction of osteosarcoma. However, the application of 4-NQO in the middle diaphysis of the femur showed a low incidence (30%). Moreover, the inducibility of DMBA, which seldom induced osteosarcoma, was up to 33.3% in the group given the chemical to the distal metaphysis. These findings indicate that the induction of osteosarcoma in the bone marrow is influenced by a variety of chemical agents, intramedullary circumstances, and/or anatomical and physiological differences of the tissues at the site of administration. In this study, the intramedullary circumstances were especially important, so that the tissue reaction to chemicals was carefully examined.

During the latent period, the three components of tissues were observed surrounding the chemicals; tissue necrosis, fibroblastic tissue, and osteofibrous or osteoid tissue. The quantitative difference of each component varied with differences in both chemicals and circumstances in the bone marrow, and was categorized as three entities (Table II).

In entity I, granuloma or connective tissues appeared around the chemicals, and sometimes gave rise to necrosis in a small amount. No osteoid tissue was observed and the relationship between the chemical and tissue seemed to be still static (Photo 4).
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In these cases, no tumor appeared or a few benign tumors, such as fibroma, were recognized. As for entity II, necrosis with the chemicals extended to some degree in the bone marrow and affected directly or indirectly the proliferating fibroblastic tissues (Photo 5). In these cases, however, a small amount of osteoid appeared occasionally outside the fibroblastic tissue. Several fibrosarcomas and occasionally osteosarcomas were observed. In entity III, the carcinogen-induced necrosis appeared in a considerable area of bone marrow, and seemed to react indirectly and successively with the osteoid or bone tissue, which proliferated surrounding the necrosis (Photo 6). In these cases many osteosarcomas were produced and chondrosarcomas appeared occasionally. Fibrosarcoma, however, was seldom observed.

In this work, the administration of 3-MC gave rise to entity I in all experimental groups. As to DMBA, entity I was found in most cases and entity II or III was observed in few cases in the distal metaphysis of the femur. Application of 4-NQO brought about entity III in the mandible and the distal metaphysis, although entity II was often induced in the middle diaphysis of the femur (Photo 7). Therefore, it was proved that even 4-NQO, which showed an excellent carcinogenicity, is not so suitable to form entity III in the diaphysis. Moreover, DMBA with a rather lower potentiality occasionally formed entity II or III in the metaphysis of the femur and the mandible (Photo 8).

Discussion

Our present results show that, of the 3 carcinogens studied, 4-NQO was by far the most effective in producing osteosarcoma, in percentage of animals responding in the anatomical sites tested (Table I). The highest incidence of osteosarcoma was found in the mandible and in the distal metaphysis of the femur, which contain abundant cancellous marrow and is the physiological growth center of the bone. In contrast the middle diaphysis of the femur was less satisfactory for osteosarcoma induction. The distal metaphysis is more abundant in cancellous marrow than is the middle diaphysis, suggesting that some kind(s) of neoplastic inductive superiority exists in the cancellous bone marrow, which could form a good repair in disturbed bone tissue.

Accordingly, we examined histological preparations of marrow from each site after the administration of carcinogen. In general, 3 different responses were recognized in the area of chemical deposition (Table I) which differed qualitatively and quantitatively. For convenience, we classified the observed tissue reaction into 3 categories (entities I, II, and III) depending on the degree of necrotic, fibroblastic, or osteoid tissue encountered. The development of each entity was directly related to the dosage and nature of the carcinogen, and to the site administered. As shown in Table II, entity I results when the effectiveness or dosage of the carcinogen is low, and usually appears in sites poor or lacking in cancellous bone marrow, such as the diaphysis of long bone. Entity II, consisting mainly of fibroblastic and sometimes osteoid tissue, results with the administration of stronger carcinogens, or with higher doses (though less than optimal level). Finally, entity III is principally osteosarcoma, with occasional chondrosarcomas and fibrosarcomas. This condition is obtained with the use of an effective chemical, adequate dose, and proper site of administration. For the latter, the metaphysis of long bones or cancellous bones, containing abundant cancellous marrow, should be chosen.

Because of a variety of chemicals and procedures employed in earlier studies, it is difficult to find a common factor with which to interpret the many findings. In our opinion, the present study offers just such a factor. Histological findings of Brunschwig and Bissell, and of Franseen et al., who succeeded in inducing fibrosarcoma in rat
femurs (probably the diaphysis), can now be equated with entity II, and the findings in our earlier report appear to be identical with entity III.

For the experimental production of osteosarcoma, it is now evident that the choice of a carcinogen, and dose and site of administration are all of great importance. Very likely, every potential carcinogen has to be examined in this manner in future studies.

In the present work, it was observed that it is much easier to induce osteosarcomas in cancellous marrow and osteogenerative growth centers than in other sites or kinds of bone marrow. This may be due to their better osteogenic reparative abilities in response to chemical injury.

Finally, it is both interesting and informative to note that clinical osteosarcoma likewise has a high rate of occurrence in similar sites in humans.

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REFERENCES


EXPLANATION OF PLATE

Photo 1. Gross appearance of osteosarcoma (7th month), induced by the administration of 4-NQO into metaphysis of the femur. The tumor proliferates over knee joint.

Photo 2. X-ray photograph of distal metaphysis of rabbit femur.

Photo 3. Photomicrograph of experimental osteosarcoma.

Photo 4. Entity I. A small amount of fibrous tissue (F) with vessels (V) appears around 3-MC (C).

Photo 5. Entity II. Bone marrow is rather disordered with DMBA (C) and necrosis (N) surrounded by fibrous tissue (F) and osteoid (O).

Photo 6. Entity III. A large amount of necrosis (N) and osteoid (O) are seen.

Photo 7. Even if 4-NQO was applied, a large amount of fibrous tissue (F) appears sometime in diaphysis of femur.

Photo 8. Application of DMBA in metaphysis of femur. Fairly extensive fibrosis (F) is found around DMBA. No necrosis is seen.
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