PROLIFERATIVE ACTIVITY OF THE EPITHELIUM OF THE RAT LARGE INTESTINE DURING CARCINOGENESIS

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The labeling index and mitotic index were studied in the epithelium of the descending colon and ileum of rats during induction of intestinal tumors with 1,2-dimethylhydrazine. As early as 1 month after the beginning of the experiments a sharp increase in the number of pathological mitoses (up to 51%) and a change in the ratio between the phases of mitosis, with marked predominance of metaphases (up to 73%) were observed in the epithelium of the descending colon; later these indices were unchanged. Starting from the third month of the experiment an increase in the labeling index (especially in carcinoma in situ) and mitotic index was observed. In the epithelium of the ileum, where tumors never develop, proliferative activity and the mitotic index were unchanged.

KEY WORDS: 1,2-dimethylhydrazine; intestinal tumors; labeling index; mitotic index; epithelium of descending colon.

Changes in proliferative activity during carcinogenesis in experiments on animals have been analyzed in only a few investigations [3, 9, 10] although dynamic studies of this type can provide much information on the role of these disturbances in the genesis of cancer. In the experimental investigations cited above only the early stages of carcinogenesis in the skin produced by carcinogens with local action were studied. The many experimental models of tumors now available, characterized by selectivity and high frequency of development of neoplasma in particular organs induced by carcinogens with resorptive action makes the systematic study of changes in the mitotic cycle of the tissues during carcinogenesis a possibility.

The object of this investigation was to study proliferative activity of enterocytes of the descending colon and to compare it with the corresponding activity in the ileum at different stages of induction of intestinal tumors with 1,2-dimethylhydrazine (DMH). These two segments of the intestinal tract were selected for investigation because tumors develop in the first of them in 100% of animals investigated, but they never develop in the second [5, 6, 7].

EXPERIMENTAL METHOD

Intestinal tumors were induced in male rats (weighing 160-180 g at the beginning of the experiment) from the Rappolovo nursery, Academy of Medical Sciences of the USSR, by subcutaneous injection of DMH once a week in a dose of 21 mg/kg [5]. Rats of the same sex and age acted as the control. The labeling index and mitotic index were used as parameters of enterocyte proliferation; in addition, the ratio between the phases of mitosis and the number of pathological mitoses were determined, i.e., the mitotic regime was analyzed. Pathological mitoses were classified in accordance with Alov's scheme [1]. The animals were killed at intervals of 1 month for a period of 6 months, when all the rats had developed multiple intestinal tumors. Material for investigation was taken 1 week after the corresponding injection of DMH at the same time of day (10-11 a.m.). To determine the labeling index, 1 h before sacrifice the rats were given an intraperitoneal injection of thymidine-H^3 (specific activity 4-10 Ci/mmole) in a dose of 1 μCi/g. The material was fixed in a mixture of alcohol, formalin, and glacial acetic acid (6:3:1). Paraffin sections were

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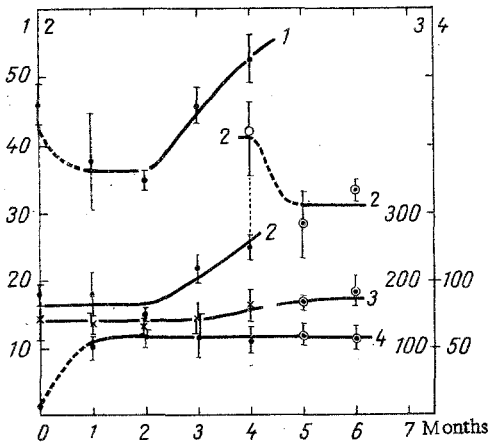

Fig. 1. Changes in labeling index in zone of proliferation (1), in labeling index (2) and mitotic index (3) throughout the crypt, and in fraction of pathological mitoses (4) at different times after beginning of DMH administration. Dot and cross represent microscopically unchanged mucous membrane; empty circle) carcinoma in situ; circle with dot) tubular adenocarcinoma. Broken line shows presumed course of curve. Abscissa, time from first injection of DMH (in months); ordinate: 1,2) labeling index (in %); 3) number of mitoses in 100 fields of vision; 4) frequency of pathological mitoses (in %).

coated with type M photographic emulsion. The number of labeled nuclei per 1000 epithelial cells in the proximal, middle, and distal parts of the descending colon and in the terminal ileum was counted in histoautoradiographs. The mitotic index was determined in the same areas by counting the number of mitoses in 100 fields of vision of the immersion system of the microscope (2 mm^2) in preparations stained with Carazzi's hematoxylin and eosin. The labeling index was determined both for the whole population of enterocytes and for the zone of proliferation, revealed by special experiments on vertical sections through the intestine by the presence of the maximal number of labeled cells forming a crypt. To count the labeling index 3 rats were used at each time, but 5 or 6 animals were used to analyze the mitotic regime. Since no difference was found in proliferative activity in the three parts of the descending colon, the corresponding numerical values were pooled. In the final stages of the experiment (5-6 months) proliferative activity was studied in tubular adenocarcinomas, which develop most frequently in the descending colon [6]; 10 tumors were studied at each time. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During the first 2 months of DMH administration the index of labeled nuclei in the epithelium of the descending colon, calculated for the whole population of enterocytes, was virtually unchanged and was equal to that for the control rats (Fig. 1). After the second month of the experiment, a clear increase was observed in the number of nuclei incorporating labeled DNA precursor. A particularly large number of labeled nuclei was observed in carcinoma in situ, against the background of a considerably increased labeling index, 4 months after the beginning of DMH administration. The number of proliferating cells in the invasive adenocarcinomas was reduced.

The labeling index when determined in the zone of proliferation during the first 2 months of the experiments was below the control; this can evidently be explained by the 10% increase in duration of the mitotic cycle compared with the control (as discovered in supplementary experiments with the aid of the curve of labeled mitoses). This decline in the number of labeled cells was followed after 2 months by a considerable increase in proliferative activity of the enterocytes.

The dynamics of the change in the number of mitoses during carcinogenesis in the descending colon largely corresponded to that for the labeling index: Initially (1 and 2 months) the mitotic index showed no marked change, but later the number of mitotically dividing cells gradually increased and was maximal in the adenocarcinomas.

In the mucous membrane of the descending colon of the control rats 4% of pathological mitoses was observed. However, by the first month after the beginning of the experiment the proportion of pathological mitoses had increased to 51.3%. Later this index remained stable at 56.5-60%. The number of abnormal mitoses in the adenocarcinomas was not reduced 2-3 months after the end of DMH administration. A wide variety of forms of pathological mitoses (bridges, delay in separation of chromosomes and their fragments, scattering of chromosomes, hollow and triradial metaphases, monocentric and multipolar mitoses, etc.) but with definite predominance of delayed separation of chromosomes and their fragments in metakinesis (12-17%) and of c-mitoses (10-20%), was observed both in the microscopically unchanged mucous membrane (at different times of treatment with DMH) and in the developing adenocarcinomas. The number of c-mitoses was particularly large in the adenocarcinomas (16-20%), evidently reflecting the intensive cell death in the tumors.

A characteristic index of the disturbance of the mitotic regime during carcinogenesis is the change in the ratio between the phases of mitosis observed during the first months of the experiment. For instance, whereas the relative number of prophases in the epithelium of the large intestine of the control rats was 26.4% and the relative number of metaphases was 56.6%, after 4 weeks of DMH treatment the percentage of prophases had fallen to 6.3 whereas the percentage of metaphases had risen to 73.7. At later stages this ratio between prophases and metaphases remained substantially unchanged.

It must be emphasized that similar changes in the mitotic regime in principle also take place in adenocarcinomas of the human large intestine [4].

During the investigation of the corresponding indices of proliferative activity of the epithelium of the ileum no distinct dynamics of the changes at different times after the beginning of DMH administration could be detected. The labeling index for the whole population of enterocytes (both in the control and at various periods of tumor induction) was between 15 and 20% (60-77% in the zone of proliferation), the mean number of mitoses per 100 fields of vision was 170, and the number of pathological mitoses did not exceed 3.5%; the ratio between the phases of mitosis was unchanged.

Considering the data on the change in proliferative activity of the epithelium of the descending colon, the region where most neoplasms developed, and the absence of any disturbance of the mitotic regime in the ileum, where no neoplasms developed, it can be postulated that the disturbance of proliferation (intensification of cell division, changes in the ratio between the phases of mitosis, the appearance of many pathological mitoses) plays an important role in malignant change.

The results of this investigation can be interpreted as follows. Stem enterocytes, acceptors of carcinogenic factors, on commencing the mitotic cycle [8] lose their ability to differentiate as a result of transformation by DMH and continue to proliferate. This situation is reflected in widening of the zone of proliferation in the intestinal crypts and an increase in the labeling index and mitotic index. In the early stages of carcinogenesis no intensification of proliferation can be detected, evidently because of the smallness of the stem enterocyte population undergoing transformation. Particularly intensive proliferation was observed in carcinoma in situ; its decrease in the large invasive adenocarcinomas is evidently connected with disturbance of cell nutrition in these tumors and the consequent ending up of many of the cells in the R_1-period.

Increased proliferative activity in the course of carcinogenesis is probably facilitated by the preliminary appearance of many pathological mitoses, which are responsible for the presence of cells with an unbalanced karyotype and evidently with increased ability to divide [2].

From a consideration of all the facts obtained in this investigation and the corresponding data in the literature it can be concluded that biological malignant transformation begins long before it is exhibited morphologically.

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