Regression and Persistence of Hyperplastic Hepatic Nodules Induced by N-2-Fluorenylacetamide and Their Relationship to Hepatocarcinogenesis

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SUMMARY

A dietary regimen containing the carcinogen N-2-fluorenylacetamide for the induction of hyperplastic hepatic nodules and hepatocellular carcinomas is presented. Administration of the carcinogen for 3 months yields a high number of nodules that regress after withdrawal of the carcinogen, and the incidence of hepatocellular carcinomas is very low. Feeding of the carcinogen for 1 additional month yields a population of nodules that persist after withdrawal of the carcinogen and are associated with a high incidence of hepatocellular carcinomas. This regimen may aid in the delineation of characteristics of nodules relevant to malignant transformation.

INTRODUCTION

In seeking to characterize the cellular alterations associated with the development of malignant tumors, many investigators have examined the effects of carcinogenic substances on target tissues long before the appearance of malignant tumors. A problem inherent in this line of research has been the difficulty in clearly defining the premalignant population in each carcinogenic system. Since malignant transformation is usually a focal occurrence, the alterations manifested by entire organs may be nonspecific, resulting from toxic effects of the carcinogen rather than its carcinogenic properties.

It has been suggested that the hyperplastic hepatic nodule induced by the administration of 2-FAA to rats is a population of hepatocytes from which the hepatocellular carcinomas derive. As such, biological alterations manifested by these nodules may afford a clearer understanding of the nature of the malignant change than would changes manifested by whole liver.

However, the majority of nodules induced by 2-FAA do not become malignant tumors. Rats that developed hepatocellular carcinomas generally developed only 1 or 2 although their livers contained many nodules during exposure to the carcinogen (1). This finding led us to formulate 3 alternative proposals that might explain the observed difference between the number of hyperplastic nodules and carcinomas: (a) the hyperplastic nodules evolve as a heterogenous population, only a small percentage of which are truly premalignant; (b) all nodules progressively alter, leading to an increasing risk for malignant transformation, which occurs randomly; (c) nodules do not change in characteristics from the time of their earliest appearance until malignant transformation randomly occurs.

In an attempt to determine which of these proposals is correct, a regimen was devised with the use of 2-FAA.

MATERIALS AND METHODS

Details of the preparation of the diet containing 2-FAA have been previously reported (5). Male, Sprague-Dawley, CFE rats (Carworth Farms, Inc., New York, N. Y.), which weighed 100 g at the beginning of the regimen, were fed the basal diet for 1 week. After this, diet containing 0.06% 2-FAA was fed for a 3-week period followed by a basal diet for 1 week. One group of rats were exposed to 3 such cycles, while another received 4 cycles.

Laparotomy was performed under light ether anesthesia. All surfaces of each lobe (except the caudate) were examined by gentle manipulation with cotton-tipped applicator sticks, soaked in warmed (37°C) 0.9% NaCl solution. When possible, nodules were measured in situ by use of a micrometer. All nodules were measured when livers were obtained at sacrifice. At that time, representative tissue from nodules was obtained and prepared by standard histological technique.

RESULTS

Three-Month Feeding (3 Cycles) (Table 1). At the termination of the 3-month (3-cycle) feeding regimen, 45 of 50 rats

Supported by USPHS Grant CA10978-01 and Damon Runyon Fund Grant DRG-1022A.

Career Scientist of the Health Research Council of the City of New York.

The abbreviation used is: 2-FAA, N-2-fluorenylacetamide.

Received June 23, 1970; accepted September 16, 1970.
demonstrated grossly visible, tan nodules, which bulged from the external and cut surface of the liver. The nodules were paler than the surrounding liver and more friable. These nodules were present uniformly in all lobes and measured from 2 to 8 mm in diameter. The average number of hyperplastic nodules present simultaneously, except for the 1 rat in which nodules regressed completely never developed hepatomas. In those rats in which hepatomas appeared, nodules were present uniformly in all lobes and measured from 3 to 9 mm in diameter. The average number of hyperplastic nodules in each liver was 15.

These 50 rats were autopsied, and 3 to 5 sections were taken per liver, which enabled us to examine approximately two-thirds of all nodules. The cells that composed these nodules were similar to those previously described (1, 5). They were enlarged hepatocytes with large vesicular nuclei and a reticulated eosinophilic cytoplasm. These nodules compressed the surrounding parenchyma. The intervening parenchyma of some but not all rats demonstrated mild bile ductular proliferation and trabecular fibrosis, which imparted a pseudocirrhotic pattern. The hepatocytes within these areas were normal in appearance.

Within 2 months of the cessation of feeding 2-FAA, only 12 of 25 randomly selected rats (45%) that were subjected to laparotomy demonstrated nodules. These were smaller in diameter than those seen 2 months before, ranging from 1 to 5 mm in diameter. Six rats that demonstrated no gross nodules were sacrificed, and 2 sections from each lobe revealed a few areas of hepatocyte hyperplasia. Six rats with nodules were sacrificed, and every nodule was composed of enlarged hepatocytes.

By 6 months after cessation of feeding 2-FAA, 2 of 25 rats (8%) contained 1 and 2 flattened, 5-mm nodules respectively. In the total 14-month period of observation, 1 of the 25 survivors of this regimen (4%) developed a solitary hepatocellular carcinoma. This tumor was considered to be a poorly differentiated hepatoma, as were all of the malignant hepatomas that developed in the 4-cycle group. The gross appearance of these carcinomas was always distinct from that of the nodules. The carcinomas were gray and/or white rather than tan and had irregular margins rather than the smooth and well-defined border of the nodules. No lesion that was judged to be a hyperplastic nodule on gross examination ever demonstrated characteristics of hepatocellular carcinoma on histological examination. There was no evidence of peripheral invasion, abnormal mitosis, or cellular atypia, and all nodules that were examined were similar in appearance. Because of this consistent correlation between gross and microscopic appearance, some nodules were scored as hyperplastic even though histological examination was not made.

The overall mortality was approximately 25%, occurring predominantly during the 3rd and 4th feeding cycles.

**Four-Month Feeding (4 Cycles)** (Table 1). At the termination of feeding after 4 months (4 cycles), 75 of 80 rats (94%) demonstrated gross hyperplastic hepatic nodules. These were also uniformly distributed through all lobes and measured from 3 to 9 mm in diameter. The average number of hyperplastic nodules was 20/liver. All 80 of these rats were examined histologically (1 section of every nodule greater than 1 mm in diameter). These nodules were composed of cells indistinguishable from those discovered at the end of the 3-month feeding.

The intervening parenchyma was qualitatively similar to that seen in the 3-cycle group.

Six months after cessation of feeding 2-FAA, 37 rats were examined. Twenty-six of these (70%) demonstrated hyperplastic hepatic nodules. Rats that demonstrated marked weight loss were sacrificed between 4 and 10 months. Several demonstrated hepatocellular carcinomas as early as 3 months after cessation of feeding of 2-FAA, but the majority of carcinomas were detected at the 9th and 10th month. Fourteen of 23 rats had hepatomas (61%) by 12 months after cessation of feeding 2-FAA.

Several findings were evident for both regimens when the same rat was examined by repeated laparotomy and autopsy. The regression of nodules following cessation of feeding occurred uniformly in all lobes. The hepatocellular carcinomas occurred in all lobes. Those few livers that did not demonstrate grossly visible nodules at laparotomy at the end of 2-FAA feeding never developed nodules throughout the 11-month period of observation. In livers in which total regression of nodules occurred, nodules did not reappear later in their course. Those rats that either never developed nodules or in which nodules regressed completely never developed hepatomas. In those rats in which hepatomas appeared, nodules were present simultaneously, except for the 1 rat in the 3-cycle group that developed a hepatocellular carcinoma.

**DISCUSSION**

The results of this regimen demonstrated a relationship between the amount of carcinogen ingested by a rat and the ultimate incidence of hepatocellular carcinomas. Furthermore, the results reinforce the existing evidence for a linear relation-
ship between the hyperplastic nodule and hepatocellular carcinoma (2). In this regimen, many nodules persisted after 4 cycles of 2-FAA feeding, and this persistence of nodules was associated with a high incidence of cancer. By contrast, after 3 feeding cycles, most of the nodules had regressed within 2 months after cessation of feeding carcinogen, and the incidence of cancer was very low.

It may be argued that some of the later nodules of this regimen represent extremely well-differentiated hepatomas rather than hyperplastic nodules. Reuber and Firminger (4) indicated that there was difficulty in differentiating hyperplastic lesions from well-differentiated tumors. However, in this regimen, we feel it is most unlikely that these late nodules are hepatomas. They demonstrated marked uniformity of gross and histological appearance and did not differ in appearance from the early nodules.

We feel that the results of this study tend to support the proposal that the hyperplastic nodules evolve as a heterogeneous population. The difference in degree of regression of the 3- and 4-cycle nodules suggests that there is a change in the character of the nodules effected by an additional month of feeding 2-FAA. This change may be highly relevant to the malignant transformation.

It is highly unlikely that the difference in incidence of malignant tumors in the 2 regimens relates simply to the difference in the number of nodules at risk at the end of the feeding period, i.e., 15 versus 20. The difference in the incidence of tumors is so great that the calculated probability that a nodule of the 4-cycle group will become a carcinoma is more than twice that for a nodule in the 3-cycle group.

We suggest that future studies with 2-FAA and other hepatocarcinogens closely correlate metabolic alterations with the amount of carcinogen ingested. Furthermore, study of the nodules that persist after cessation of feeding 2-FAA rather than study of early nodules may be more fruitful in the delineation of changes critical for the development of malignant tumors.

REFERENCES
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