Modifying Role of Partial Hepatectomy and Gonadectomy in Ethylnitrosourea-induced Hepatocarcinogenesis

S. D. Vesselinovitch, L. Itze, N. Mihailovich, and K. V. N. Rao

ABSTRACT

The modifying effect of partial hepatectomy and gonadectomy upon carcinogenesis in general and hepatocarcinogenesis in particular has been evaluated in 6-week-old C57BL/6J × C3HeB/FeJ F1 mice. Ethylnitrosourea (60 μg/g body weight) was administered once after sham hepatectomy or 48 hr following partial hepatectomy at which time DNA synthesis in the regenerating liver was 30-fold higher than in the sham-hepatectomized mice. Six days later, one-half of the partially hepatectomized and one-half of the sham-operated mice were gonadectomized. Survivors were killed at 90 weeks of age. The incidence of tumors has been evaluated at all tissue sites. The sex hormonal environment of the hosts was assessed by histological evaluation of kidneys and adrenal glands. This evaluation showed that following gonadectomy, the morphology of the above tissues and consequently the hormonal environment shifted from that observed in the intact mice towards one characteristic of the opposite sex, assuming an intermediate state. Although primary tumors developed at several sites, partial hepatectomy and gonadectomy modified primarily the development of liver tumors. Thus, partial hepatectomy enhanced significantly hepatocarcinogenesis in male but not in female mice, resulting in an increased difference in the incidence of liver tumors between the two sexes. Gonadectomy of males abolished the enhancing effect of partial hepatectomy upon ethylnitrosourea hepatocarcinogenesis while ovariectomy potentiated development of liver tumors, leading to a similar incidence of liver tumors in both castrates. Modulating effects of hepatectomy and gonadectomy operated by influencing the incidence of benign rather than malignant liver tumors. The ovariectomy of partially hepatectomized females also enhanced kidney tumorigenesis. Data suggested that since enhancing effect of partial hepatectomy was associated with increased macromolecular (DNA) synthesis at the time of carcinogenic treatment while the other half were only sham gonadectomized. Animals were kept at 21 °C environmental temperature and were given Rockland mouse diet and water ad libitum. Animals were weighed at 2-week intervals and at autopsy. The experiment was terminated at 90 weeks. Specimens were taken from all tissues which were stained by hematoxylin and eosin for histological evaluation, and the incidence of tumors has been evaluated at all tissue sites.

INTRODUCTION

Warwick (20), Chernozemski and Warwick (3), Hollander and Bentvelzen (8), and Lane et al. (10) showed the enhancement of induced hepatocarcinogenesis in adult mice by partial hepatectomy preceding carcinogenic treatment. Their studies indicated that the degree of hepatocarcinogenesis was dependent upon the rate of macromolecular replication, specifically DNA, at the time of carcinogenic treatment. The incidence of liver tumors could also be influenced by hormonal environment of the host even when the change of this environment occurs following carcinogenic treatment (18). The present studies were designed to evaluate the modifying effects of partial hepatectomy and gonadectomy upon induced tumorigenesis in general and hepatocarcinogenesis in particular. ENU has been chosen as the carcinogen because under in vivo conditions it undergoes nonenzymatic, heterolytic decomposition with a half-life of approximately 8 min (6), generating an ethyl cation as the ultimate reactant in all tissues. The extremely short half-life of ENU in vivo permits well-defined timing of treatment in relation to partial hepatectomy and gonadectomy, offering the possibility to explore interaction of the 2 procedures upon the outcome of carcinogenesis.

MATERIALS AND METHODS

C57BL/6J × C3HeB/FeJ F1 (hereafter called B6C3F1) mice of both sexes were used in this study. The C57BL females and C3H males were purchased from The Jackson Laboratory, Bar Harbor, Maine. Animals were bred in our laboratory when 10 to 12 weeks of age. Their offspring were used in the current study. At 6 weeks of age, mice were either partially hepatectomized or sham operated (7) and were treated 48 hr later with a single i.p. injection of ENU (60 μg/g body weight). The carcinogen has been synthesized by Dr. J. M. Rice of the National Cancer Institute and was dissolved [0.6% in trioctanoin (Eastman Kodak Co., Rochester, N. Y.)] shortly before its administration. The experimental design is summarized in Table 1. One-half of the two-thirds-hepatectomized and sham-operated animals were gonadectomized 1 week following carcinogenic treatment while the other half were only sham gonadectomized. Animals were kept at 21 ° environmental temperature and were given Rockland mouse diet and water ad libitum. Animals were weighed at 2-week intervals and at autopsy. The experiment was terminated at 90 weeks. Specimens were taken from all tissues which were stained by hematoxylin and eosin for histological evaluation, and the incidence of tumors has been evaluated at all tissue sites.

A sexual dimorphism has been described for the mouse kidney and the submaxillary salivary gland (5, 18). Morphology of the kidneys was used for the assessment of androgenic and/or estrogenic stimulation of the host. Thus, cells lining Bowman capsule are cuboidal in the intact males (androgenic hormonal environment) and flat in the intact females (estrogenic hormonal environment). Following gonadectomy, lining cells...
assume morphology intermediate to those observed in the intact mice (castrate hormonal environment). In addition to these morphological changes in the kidney, the adrenal gland in gonadectomized mice shows hyperplasia of the β-type cells characterized by foamy to clear (lipid-laden) cytoplasm. These cells are either distributed subcapsularly or present through the entire depth of the cortex, and their presence is readily identified. The hyperplasia of β-cells is associated with increased secretion of sex steroids.

RESULTS

Table 2 lists the incidence of tumors observed in the experimental and control groups and the average age at which the animals died, including those killed at 90 weeks of age. The animals tolerated the treatment well as indicated by the high ratio of effective/original number of mice (Table 2, Column 3). Although a great variety of tumors developed, 6 of 8 ENU-treated groups showed similarly high survival (83 to 89 weeks). The ENU treatment affected the development of tumors in lungs, Harderian glands, forestomach, lymphoreticular tissues, kidneys, and livers.

Lung Tumors. Pulmonary tumors, primarily lung adenomas, were readily induced by ENU with an average incidence of 90% in males and 85% in females with multiplicities of 6.0 and 4.8 for males and females, respectively. Partial hepatectomy and gonadectomy had no effect on incidence of these tumors. The multiplicity of the lung tumors was somewhat higher in the gonadectomized groups (7.4 and 7.5 in Groups 9 and 10, respectively) and in groups which were partially hepatectomized and gonadectomized (8.7 and 10.0 in Groups 7 and 8, respectively).

Harderian Gland Adenomas. The tumors were seen in 27 and 30% of males and females exposed to ENU, respectively. Neither gonadectomy nor partial hepatectomy showed any significant effect upon their incidence.

Forestomach Tumors. These tumors were observed with similar frequencies in all ENU-treated groups regardless of surgical manipulations. In the whole ENU series, such tumors were observed in 27 male and 48 female mice (13.6% versus 21.9%; p < 0.05).

Lymphoreticular Tumors. These were developed in 15% of the intact males and 28% of the intact females treated with ENU. In other groups, females showed repeatedly the same or lower incidence of these tumors in relation to similarly treated males.

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective no.</th>
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<th>Gonadectomy</th>
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* Indicated groups were partially (two-thirds) hepatectomized 48 hr before carcinogenic treatment.

** ENU (0.6%) in trioctanoin (60 μg/g body weight) was injected i.p. 48 hr following partial hepatectomy.

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Kidney Tumors. Papillary and tubular adenomas and adenocarcinomas were induced only marginally in mice treated with ENU. Kidney tumors have been enhanced significantly only in ENU-treated females which underwent both partial hepatectomy and ovariectomy (Group 6, 5 of 57; p < 0.05).

Ovarian Tumors. Tubular adenomas and granulosa cell tumors were in controls (Group 2, 1 of 96, 1%); sham-hepatectomized, ENU-treated mice (Group 4, 10 of 54, 19%); and partially hepatectomized, ENU-treated females (Group 6, 7 of 57, 12%).

Liver Tumors. The incidence of animals bearing either benign or malignant liver tumors is presented for each group in Table 2, Column 8. Animals which were exposed to ENU in the absence of partial hepatectomy and gonadectomy developed liver tumors in 25% of males (Group 3 versus Group 1; p < 0.01) and 7.4% of females (Group 2 versus Group 4; p > 0.05). When the administration of carcinogen was preceded by partial hepatectomy (Groups 5 and 6), the incidence of liver tumors increased significantly in males (Group 5, 46.4% versus Group 3, 25.0%; p < 0.05) but not in females (Group 6, 11% versus Group 4, 7%; p > 0.05). It is important to emphasize, however, that the enhancement of hepatocarcinogenesis in Group 5 has been observed even though their average age at death was 16 weeks shorter than the age at death of non-hepatectomized, ENU-treated mice (Group 3). This indicates also, therefore, an accelerating effect of precedential partial hepatectomy upon ENU-induced hepatocarcinogenesis.

The orchidectomy of partially hepatectomized and ENU-treated mice excluded enhanced development of liver tumors by partial hepatectomy (Group 7, 27.5% versus Group 5, 46.4%; p < 0.05). Since both of these groups showed similar longevity (Group 7, 72 weeks; Group 5, 69 weeks), the differential tumor response could be related directly to modifying effects of partial hepatectomy and orchidectomy. The ovariec-
tomy of partially hepatectomized and ENU-treated females potentiated development of liver tumors (Group 8, 34.0% versus Group 6, 11%; p < 0.01). Orchidectomy of mice which received only ENU treatment without partial hepatectomy (Group 9) excluded hepatocarcinogenic effect of ENU since their tumor response was similar to that observed in the non-treated controls (Group 1, 4% versus Group 9, 6%; p > 0.05). The ENU-treated females which were ovariectomized showed marginal incidence of liver tumors.

Table 3 presents morphology of liver tumors and the multiplicity and weight of livers bearing such tumors. The liver tumors were classified into hepatocellular adenomas and carcinomas according to recently published criteria (19). The objective of such data presentation was to find out whether the above experimental conditions influenced the incidence of both benign and malignant liver tumors and whether the latter 2 parameters were affected by experimental manipulations. From the inspection of Table 3, it is obvious that partial hepatectomy and gonadectomy mainly influenced development of hepatocellular adenomas. Their incidence was enhanced by hepatectomy (Group 5, 18/56 versus Group 3, 7/52; p < 0.05) and excluded by orchidectomy (Group 7, 5/40 versus Group 5, 18/56; p < 0.05). The incidences of hepatocellular carcinomas were not, however, affected by such manipulations. Similarly, the ovariec-
tomy in conjunction with partial hepatectomy enhanced development of adenomas but not of carcinomas (Group 8, 15/50 versus Group 4, 4/54; p < 0.01). Thus, it is apparent that partial hepatectomy and/or gonadectomy modified the outcome of hepatocarcinogenesis only by influencing the development of benign liver tumors, which may represent a hormone-dependent cell population.

Multiplicity of liver tumors was constant throughout all ex-

Table 4
Frequency of mice showing hyperplasia of β-cells in the adrenal glands and sex-associated morphology of Bowman’s capsule

<table>
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<th>GX</th>
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* MHE, male hormonal environment; FHE, female hormonal environment; CHE, castrate hormonal environment; PH, partial hepatectomy performed 48 hr before administration of ENU; ENU, single i.p. administration of ENU (80 µg/g body weight); GX, gonadectomy performed 6 days following administration of ENU.
perimental series (Table 3, Column 6). Tumor growth as measured by weight of tumor-bearing livers was uniform in all groups.

Table 4 presents the incidence of mice showing hyperplasia of beta-cells of the adrenal cortex and gives for each experimental group the frequencies of mice showing cuboidal, flat, and intermediate morphology of cells lining Bowman capsule. Data show that in the gonadectomized animals, there was an increased incidence of mice having hyperplasia of beta-cell suggesting an increase in the secretion of sex steroids. Gonadectomized mice also showed a change in the morphology of cells lining Bowman capsule. These cells lost their morphology characteristic for the sex of intact (nongonadectomized) mice and assumed an intermediate appearance suggesting lack of characteristic for the sex of intact (nongonadectomized) mice groups.

Table 4: Incidence of mice showing hyperplasia of beta-cells of the adrenal cortex and giving for each experimental group the frequencies of mice showing cuboidal, flat, and intermediate morphology of cells lining Bowman capsule. Data show that in the gonadectomized animals, there was an increased incidence of mice having hyperplasia of beta-cell suggesting an increase in the secretion of sex steroids. Gonadectomized mice also showed a change in the morphology of cells lining Bowman capsule. These cells lost their morphology characteristic for the sex of intact (nongonadectomized) mice and assumed an intermediate appearance suggesting lack of characteristic for the sex of intact (nongonadectomized) mice groups.

Thus, there was a shift from the predominantly male hormonal environment in Groups 1, 3, and 5 to the castrate state in Groups 7 and 9 and from predominantly female hormonal environment in Groups 2, 4, and 6 to the castrate state in Groups 8 and 10. This shift from specific sex hormonal environment to intermediate or castrate hormonal state was associated with shift in tumor incidence (Tables 2 and 3). Orchidectomized males and ovariectomized females showed similar incidence of liver tumors.

**DISCUSSION**

The presented data showed that partial hepatectomy and gonadectomy modified the incidence of tumors at only 2 sites, kidney and liver. In the case of kidney carcinogenesis, an enhancement has been observed only in ENU-treated females which underwent both partial hepatectomy and ovariectomy, which is in agreement with the previous report (17). A more complex situation, however, exists regarding the modification of hepatocarcinogenesis. Thus, partial hepatectomy preceding the carcinogenic treatment significantly enhanced the incidence of liver tumors above that observed in the intact mice, the manifestation of which was dependent upon hormonal environment of the host. The enhanced effect of partial hepatectomy upon hepatocarcinogenesis has been observed only in the male series. This may be interpreted as being associated with differential degree of the "initiating" effects of ENU in 2 sexes. However, the indirect evidence that these effects were similar in both sexes could be deducted from the inspection of the observed incidences of liver tumors in Groups 7 (males) and 8 (females). These groups were partially hepatectomized, ENU-treated, and 6 days later gonadectomized. Under those conditions, both groups responded similarly to the carcinogenic treatment (orchidectomized males, 27.5%; ovariectomized females, 34.05%; p > 0.05). Also, no statistical difference in the incidence of liver tumors has been observed between nonorchidectomized males and ovariectomized females (Group 5, 46.4% versus Group 8, 34.0%; p > 0.05). Thus, it appears that the shift from the predominantly male or female hormonal environment to the castrate state was equally permitting or promoting the emergence of liver tumors from the originally initiated cells (16). It appears that under the experimental conditions, castrate hormonal state in male and female hormonal environment in nonovariectomized females was capable of overriding the enhancing effect of partial hepatectomy upon ENU "initiation" of hepatocarcinogenesis. Complementary to this, the male hormonal environment of nonorchidectomized males and the castrate hormonal state in females permitted enhanced development of liver tumors due to partial hepatectomy.

Studies of Peraino et al. (11) showed that phenobarbital effectively enhanced 2-acetylaminofluorene-induced hepatocarcinogenesis in rats. Pitot (12) demonstrated that the same agent was capable of promoting hepatocarcinogenesis initiated by diethylnitrosamine in partially hepatectomized rats. Tannenbaum and Silverstone (15) presented evidence that the level of caloric intake can effectively modulate mouse hepatocarcinogenesis when present during developing or promoting phase of carcinogenesis. Armuth and Berenblum (1, 2) were able to observe the promoting effect of phorbol esters upon the development of liver tumors in 2-acetylaminofluorene- and dimethylnitrosamine-treated mice. The present studies further contribute to the 2-stage concept of hepatocarcinogenesis by showing that the type of hormonal environment present during promotion may modulate significantly the outcome of hepatocarcinogenesis.

In our related biochemical studies, we observed that two-thirds partial hepatectomy of 6-week-old B6C3F1, mice resulted in an increase in DNA synthesis characterized by 3 major peaks occurring at 30, 42, and 72 hr following surgery (9). The first peak of DNA synthesis was preceded by RNA and protein synthesis (12 and 6 hr, respectively). At the time of ENU administration (48 hr after partial hepatectomy), DNA synthesis was approximately 30-fold higher than its background rate. Thus, similar to the observations of Chernozemski and Warwick (3), Hollander and Bentvelzen (8), and Lane et al. (10) in mouse studies and those of Craddock (4) and more recently of Rabes et al. (13) in rats, increased DNA synthesis triggered by partial hepatectomy was associated with an enhancement of hepatocarcinogenesis. Recently, we reported that the administration of the same dose of ENU (60 μg/g body weight, once) to 15-day-old intact males resulted in significantly higher incidence of liver tumors than in similarly treated 42-day-old, partially hepatectomized, male mice (98% versus 46%; p < 0.001) even though in both instances DNA-synthetic activity was of similar high level (16). However, differential effect of carcinogenic treatment on DNA synthesis has been observed between these 2 groups. Thus, while ENU administration led to immediate but transient inhibition of DNA synthesis in partially hepatectomized mice, it had no such effect on 15-day-old infants. This suggested that kinetics of DNA synthesis following carcinogenic treatment are of crucial importance regarding degree of enhancement of hepatocarcinogenesis. Recently, we stated that "Because the degree of residual biochemical lesion inflicted by carcinogen [e.g., 06 ethylation of guanine (14)] depends upon the degree of its fixation and the rate of its [error-free] repair . . . it may be speculated that relatively high DNA synthetic activity after carcinogenic treatment favors fixation over repair" and thus leads to an even more effective carcinogenesis (16). The most recent studies of Rabes et al. (13) pointed out that not only the extent and site of DNA alkylation but also the specific position of a cell in the cell cycle during carcinogen exposure might be of crucial importance for initiation of carcinogenesis. Our observations suggest that due consideration should also be given to the degree of DNA synthesis which occurs following administration of the carcinogen.

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REFERENCES

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