ABSTRACT

The potential of X-rays to induce preneoplastic lesions in the rat liver was studied in order to clarify the reason why X-rays are ineffective in inducing hepatocellular carcinoma in this animal. Male newborn rats at 8 or 22 days of age received whole body X-ray irradiation of 100 to 400 rads. After weaning they were fed either basal diet or a diet containing 0.05% phenobarbital as a promoter. X-rays induced numerous adenosine triphosphatase-deficient islands appearing in the liver by wk 22 of age. However, they were generally small, γ-glutamyl transpeptidase-negative, and did not clearly respond to the promoting stimulus of phenobarbital. No hepatic tumors were observed by 22 mo after radiation, even in phenobarbital-treated animals. Thus the X-ray-induced enzyme-altered islands differ somewhat qualitatively from those induced by potent hepatic carcinogens and their preneoplastic potential if at all present may be very low. Similarities between these X-ray-induced lesions and some types of spontaneous enzyme-altered islands are pointed out.

INTRODUCTION

The oncogenic effects of external radiation including X-rays are well established both in animal experiments and by observations of human occupational and accidental carcinogenesis (1). Experimentally X-irradiation induces cancers in various species of animals and in various organs including skin, lung, gastrointestinal tract, genitourinary system, hematopoietic system, and skeletal and connective tissues (1, 2). In the mouse, X-rays induced overt carcinomas but rather of hyperplastic nodules. Oncogenicity of external radiation, even in phenobarbital-treated animals. Thus the X-ray-induced enzyme-altered islands differ somewhat qualitatively from those induced by potent hepatic carcinogens and their preneoplastic potential if at all present may be very low. Similarities between these X-ray-induced lesions and some types of spontaneous enzyme-altered islands are pointed out.

MATERIALS AND METHODS

Male Wistar-Ms rats bred in the Animal Breeding Facility of the National Institute of Radiological Sciences were used. Newborn animals at day 8 or 20 were given whole-body X-irradiation of 50, 100, and 400 rads (200 kVp; 20 mA; half-value layer, 1.2 mm Cu; 72 rads/min). At wk 22 of age they were weaned and thereafter fed a basal diet (CE-II; CLEA Japan Inc., Tokyo) or a diet containing 0.05% PB (Iwaki-seiyaku Co., Tokyo) as a promoter. Some of the animals were sacrificed at 22 wk of age and the remaining animals were maintained on either basal or PB diet for long-term observation.

The livers were excised immediately after sacrifice and sliced at intervals of 5 mm. The slices were arranged side by side on pieces of filter paper and frozen on dry ice. Using a cryostat, 12 sets of serial sections were made at a thickness of 12 μm taken at 1-mm steps through the tissue blocks, one section measuring 4.5 to 5.0 cm² in area. One of the serial sections was fixed in cold formal calcium overnight and stained for canalicular ATPase (15), and size and number of ATPase-deficient islands larger than 50 μm in greatest diameter were measured utilizing an eye piece grid at the microscope. The remaining 2 sections were air dried and stained for GGTase (16) or G6Pase (15) for comparison.

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A total of 24 animals kept on PB diet and 3 animals kept on basal diet sacrificed or dying spontaneously during the 12- to 22-mo period after irradiation were examined for hepatic tumors.

RESULTS

The animals receiving 400 R of X-ray were slightly retarded in growth, at 22 wk of age being about 90% of normal weight and chemical hepatocarcinogenesis the effect of initiation and promotion can be measured by scoring the number and size of enzyme-altered islands, which are focal proliferations of initiated cells with distinct enzymatic deviation from normal hepatocytes (11, 12). The purpose of the present experiment was to assess the initiating activity of X-rays by reference to development of EAI's and also on the assumption that deficiency in promoting potential might underly the lack of carcinoma induction after X-irradiation to test whether tumors arise with the aid of the hepatopromoter PB. It was shown previously that the carcinogenicity of chemicals which have initiating activity but are deficient in promoting activity may be demonstrated by the use of promoter acting on EAI's to bring about tumor development (13, 14).

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2 The abbreviations used are: EAI, enzyme-altered island; PB, phenobarbital; ATPase, adenosine triphosphatase; GGTase, γ-glutamyl transpeptidase; G6Pase, glucose 6-phosphatase; AAF, acetylaminofluorene.
apt to die after 1 yr because of infection. However, the livers were normal in both size and macroscopic appearance. The animals receiving 50 and 100 R of X-ray did not demonstrate appreciable growth retardation.

Enzyme histochemical study revealed a large number of ATPase-deficient EAIs (Figs. 1 and 2) in all the livers of X-irradiated animals at 22 wk of age. The average numbers of EAI/cm² of X-ray doses in animals irradiated at day 8 or 20 of age and subsequently kept on basal diet are shown in Tables 1 and 2, respectively. There was a roughly dose-related increase of EAIs in both animal groups, although irradiation at day 8 of age was much more effective than at day 20 of age in inducing EAIs. All the EAIs were small, less than 500 μm in diameter. Almost all the ATPase-deficient EAIs were GGTase negative and more than 90% of them were moderately or weakly positive for G6Pase activity (Figs. 7 and 8). GGT-positive EAIs were found very rarely (less than 0.1/cm²) in both PB-promoted and non-promoted groups (Figs. 5 and 6). Rare spontaneous EAIs were observed in nonirradiated control animals, these generally being very small, less than 250 μm in diameter and usually negative both for ATPase and GGTase activities. Very exceptionally spontaneous EAIs were GGTase positive.

In PB-fed animals, EAIs were similar in number, size, and enzymatic features to those seen in animals kept on basal diet. In PB-fed animals, however, the impairment of canalicular ATPase activity of the mid- to central portion of normal hepatic lobules (Fig. 3), a common effect of PB, interfered with accurate scoring of the small EAI as seen in this experiment.

Histologically EAIs had minimal structural and/or cellular atypism. They were occasionally discernible, however, when they showed some irregularity in structural arrangement and/or clear cytoplasm and/or slight difference in nuclear size, usually smaller than those of normal hepatocytes (Fig. 4).

Neither gross tumors nor nodular lesions were observed in the livers of the 27 rats autopsied during 12 to 22 mo after radiation. However, 4 skin tumors, 1 colon, and 1 lung carcinoma were detected.

**DISCUSSION**

The present experiments have shown that X-rays are capable of inducing ATPase-deficient EAIs in the rat liver. Thus, so long as ATPase-deficient islands are indiscriminately regarded as preneoplastic lesions, the conclusion drawn must be that X-rays have initiating activity in the rat liver. The ATPase-deficient islands observed here are, however, somewhat different from those commonly seen in the liver during chemical carcinogenesis; the X-ray-induced lesions increase in size very slowly, are negative for GGTase and positive for G6Pase activities, do not clearly respond to the promoting stimulus of PB as seen in diethylnitrosamine- or 3'-methyl-4-(dimethylamino)azobenzene-induced EAIs (11), and do not give rise to nodules or carcinomas within a 22-mo observation period, even in the presence of a promoter. Their preneoplastic potential may therefore be very low if present and extended life span observation is necessary to clarify their true nature and histogenetic fate.

It has been repeatedly pointed out that EAIs induced by chemical carcinogens are by no means homogenous populations (12, 18, 19). Histologically they are classified at least into 3 categories, clear, basophilic, and eosinophilic foci (20, 21). Enzyme histochemically their heterogeneity is more remarkable. Although elevation of GGTase and deficiency in ATPase and G6Pase activities have been commonly described as marker phenotypes of preneoplastic EAIs, lesions expressing all these 3 enzyme alterations comprise only part of the total number of EAIs and in practice 7 different types of EAI have been distinguished according to the presence of these particular markers, alone or in combination (12, 19). Since these characteristic enzyme deviations are usually seen more distinctly and more constantly combined in larger or advanced lesions including carcinomas (18, 19) it is probable that EAIs with different phenotypic features have different neoplastic potential to give rise to true cancers in the presence or absence of further carcinogenic or promoting stimuli.

An important question which has not yet been answered is whether all EAIs can be considered as preneoplastic regardless of degree. The X-ray-induced EAI may offer a useful model system to scrutinize this problem since they appear to be a type of EAI with minimal preneoplastic or neoplastic potential, if any.

The X-ray-induced EAIs are reminiscent of a class of spontaneous EAIs developing in untreated old rat liver (4) (see Figs. 9 and 10). The elucidation of the nature and etiology of spontaneous EAIs is important for estimating the carcinogenic or promoting effects of environmental compounds. Recently similarities between spontaneous EAIs and carcinogen-induced ones with regard to their responsiveness to promoters have been emphasized (22, 23). However, spontaneous EAIs are also heterogeneous entities which may differ as populations from carcinogen-induced EAIs; they are mostly small in size, show only one of 3 enzyme markers (22), and do not clearly respond to PB (23, 24). Thus X-ray-induced EAIs share similarities with the ATPase-deficient, GGTase-negative type of spontaneous EAIs. The elucidation of the nature of X-ray-induced EAIs may therefore also contribute to the understanding of the nature and etiology of spontaneous EAIs.

Enomoto et al. (25) reported recently that X-irradiation induced GGTase-positive islands in rats, quite in contrast with our results. However, since they used acetylaminofluorene as part of the Solt-Farber selection procedure (26) adapted, it appears highly probable that what they observed were AAF-initiated islands,
not X-ray induced; 0.02% AAF combined with chemical partial hepatectomy by CCl₄ may well induce islands of countable size in 24 days (they waited for an interval of 10 days after 2 wk of AAF treatment). Indeed, they described the presence of a considerable number of GGTase-positive islands in control (no X-irradiation) animals. X-irradiation may have acted as a co-initiating factor for AAF in their experiment.

REFERENCES


*K. Enomoto, personal communication.
Figs. 1 and 2. ATPase-deficient islands (arrows) developed in the liver of a rat X-irradiated (400 R) at day 8 of age and kept on basal diet. ATPase reaction and hematoxylin, × 32 and 80, respectively.

Fig. 3. The liver of a rat X-irradiated (400 R) at day 8 of age followed by a PB diet. Note marked impairment of canalicular ATPase activity in the centro- to midlobular area and a few ATPase-deficient islands (arrows) similar in size to those shown in Fig. 1. ATPase reaction-hematoxylin, × 32.

Fig. 4. Histological features of an X-ray-induced EAI. Note thickly proliferated small hepatocytes with rather pale cytoplasm but with almost no nuclear atypism. Hematoxylin-eosin, × 80.

Figs. 5 and 6. An EAI negative for ATPase and positive for GGTase activity seen in the liver of a rat x-irradiated (400 R) and kept on basal diet. ATPase reaction-hematoxylin and GGTase reaction, respectively; serial section, × 80.
Figs. 7 and 8. An EAI weakly positive (±) for G6Pase and negative for ATPase (not shown) and GGTase activity found in the liver of a rat X-irradiated (400 R) and kept on a PB diet. The presence of GGTase-positive hepatocytes in the perportal area is a physiological finding related to aging (27). G6Pase and GGTase reaction, respectively; serial section, x 32.

Figs. 9 and 10. ATPase- and GGTase-negative EAIs spontaneously developed in untreated old rat liver. The ATPase-negative EAIs are indicated by arrows in Fig. 9. The appearance of GGTase-positive cells in perportal areas is a physiological phenomenon related with aging (27). ATPase reaction-hematoxylin and GGTase reaction, respectively; serial section, x 32.
Induction by X-Irradiation of Adenosine Triphosphatase-deficient Islands in the Rat Liver and Their Characterization

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