Promoting Effect of Nicotinamide on the Development of Renal Tubular Cell Tumors in Rats Initiated with Diethylnitrosamine

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ABSTRACT

Nicotinamide administered in the drinking water of male Fischer 344 rats increased the number of renal tubular cell tumors of rats treated with an i.p. injection of diethylnitrosamine (DEN) (25-mg/kg body weight). The incidence of kidneys with tumors in rats treated with DEN alone was 5%. In rats which received DEN and then were promoted with either 30 or 6.7 mm nicotinamide in their drinking water, the incidence of kidneys with tumors rose to 59 and 28%, respectively. Rats which were on 30 mm nicotinamide but did not receive DEN had no kidney tumors present. These results show that nicotinamide promoted DEN-induced renal tubular cell tumorigenesis.

INTRODUCTION

The concept that certain agents act as tumor promoters was described by Berenbrink in 1941 (2). He demonstrated that the alternate application of croton oil and benzo(A)pyrene increased the incidence of papillomas and carcinomas of the epidermis. Mottram was the first to describe the two stage carcinogenesis protocol when he obtained skin tumors after a subcarcinogenic administration (26). The concept of initiation and promotion was thought originally only to be applicable to the skin, but subsequently it has been extended to many other organ systems.

The initiation/promotion model of carcinogenesis has been extended to the bladder (9), liver (8, 30, 31), lung (52), intestine (32, 37), and thyroid (15). Recently there have been reports in the literature about agents which promote renal tubular cell tumors. The agents which have been suggested to be renal tumor promoters were β-cyclodextrin (14), basic lead acetate (13) and sodium arsenite (45). Both β-cyclodextrin and basic lead acetate injure the proximal convoluted tubules (13, 14). The effect of sodium arsenite on renal tubule cells was not described (45).

There have been numerous reports on the effects of various compounds on the development of renal tumors in rats treated with DMN (7, 18, 19, 27, 48, 51), streptozotocin (22, 36, 45), EHEN (12–14) and a few other compounds (3, 5, 23, 38, 43, 47). There have been a number of reports of DEN’s tumorigenicity on the kidney (24, 25, 41, 44).

Nicotinamide has been shown to be noncarcinogenic in life long administration to mice (49). In combination with streptozotocin, nicotinamide increased the incidence of pancreatic tumors but decreased the incidence of renal tumors (36). Nicotinamide increased the incidence of pancreatic tumors when administered concurrently with heliotrine (40). In another study, pretreatment with nicotinamide enhanced DEN’s ability to induce renal tumors (41). Nicotinamide also had a protective effect on brachon fenn induced carcinogenicity (29).

It has been demonstrated that rat hepatocytes acquire fetal properties and lose adult phenotypic markers as a consequence of tissue culture (46). Recently we showed that nicotinamide prevented the increase in gamma glutamyltransferase, a fetal liver marker, in cultured adult rat hepatocytes (39). Nicotinamide has also been shown to maintain cytochrome P450 levels in isolated rat hepatocytes and also increased unscheduled DNA synthesis in cultured hepatocytes (1, 28). Thus it appeared that nicotinamide was able to maintain many differentiated functions of hepatocytes which were lost during tissue culture. This experiment was designed to determine whether nicotinamide was a liver tumor promoter. Contrary to our expectations our findings demonstrate that nicotinamide promoted the development of renal tumors.

MATERIALS AND METHODS

Animals. Male Fischer 344 rats weighing approximately 150 g (60 days old) were obtained from Charles River Breeding Laboratories (Wilmington, MA). They were housed in a temperature-controlled room with a 12-hr light-dark cycle. Autoclavable Laboratory Chow 5010 (Ralston Purina Co., St. Louis, MO) and water were provided ad libitum.

Initiation/Promotion Protocol. The initiation/promotion protocol designed by Pitot et al. (30) with phenobarbital was used. The experimental protocol is shown in Chart 1. The rats were subject to a 70% partial hepatectomy by the method of Higgins and Anderson (16). The rats were divided into 5 groups with 10 rats/group, except for Group 3 which had 15 animals. Group 1 received an i.p. injection of DEN (25 mg/kg body weight) (Eastman, Rochester, NY) in H2O 24 hr posthepatectomy. Group 2 received an i.p. injection of H2O 24 hr posthepatectomy, and then 2 weeks later, the animals were placed on 30 mm nicotinamide (Sigma Chemical Co., St. Louis, MO) in their drinking water. Group 3 was identical to Group 1, except that 2 weeks after the hepatectomy, the rats were placed on 30 mm nicotinamide in their drinking water. Group 4 was the same as Group 3, except the concentration of nicotinamide was 6.7 mm. Only animals surviving longer than 15 months were used. The animals were weighed monthly. If weight loss and a palpable mass were present, then that animal was sacrificed. All surviving animals were sacrificed at 20 months. A complete necropsy was performed. All tissues which appeared grossly abnormal were examined histologically. The liver and both kidneys were fixed in 10% buffered formalin, and a single hematoxylin-eosin paraffin section was made. The kidney section was taken midline vertical. Electron microscopy was performed on representative tumors as described previously (20).

Statistical methods used were either a Students’ t test or x2 test.
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RESULTS

Growth. The mean weights of the rats at various time points are shown in Chart 2. The rats on high-dose nicotinamide (Groups 2 and 3) have a significantly reduced growth rate, and also, the final body weight was significantly different from the rats in Group 1 (p < 0.01). The final mean body weight and growth rate of the animals in Group 4 are not significantly different from those of Group 1.

Incidence of Renal Tumors. Table 1 shows the number of kidneys with renal tubular cell tumors. No mesenchymal tumors were found in any of the groups. In Group 1, one renal tumor of 20 kidneys (5%) was found at the time of sacrifice (20 months). In Group 2, no renal tumors were found. In Group 3, 13 of 22 kidneys (59%) had renal tumors. This incidence was significantly different from Group 1 (p < 0.005). The first kidney with a renal tumor in Group 3 was found at 16 months. Three renal tumors were found at 17 months, and 4 more renal tumors were found at 18 months (data not shown). The rest of the renal tumors were discovered at the time of sacrifice (20 months). Table 1 shows that 5 of 18 kidneys (28%) had renal tumors in Group 4. Two kidneys in Group 4 were found at 17 months to have renal tumors. The other renal tumors were found at the time of sacrifice (data not shown).

Macroscopic Findings. Most of the renal tumors in Groups 1, 3, and 4 were round and solid. The tumors arose in the cortex, but the large tumors extended into the medulla. No tumors were found in the pelvis. Many of the tumors, primarily the larger ones, showed areas of hemorrhage and necrosis.

Microscopic Findings. Fig. 1 shows a representative light micrograph of a renal tubular cell tumor. Histologically, the renal tumors were composed of tubular structures, except for a few tumors which appeared less differentiated where structures other than tubular were seen. The majority of the tumors had cells with large round nuclei and prominent nucleoli. The cytoplasm in all the renal tumors was basophilic. The microscopic morphology did not differ in large or small tumors, except that the larger tumors usually had areas of hemorrhage and/or necrosis. Compression of normal tissue was seen in many of the tumors, but invasion was seen in very few tumors, and only one obvious metastasis to the liver was found.

Histological findings in nontumorous areas of the kidneys of each group appeared the same. The kidney histology appeared to have no apparent pathology, thus demonstrating that no toxic effect was induced by nicotinamide. No cystic dilatation of tubules, tubular casts, or interstitial fibrosis was seen in the groups treated with nicotinamide. In all groups, there were clusters of hyperbasophilic proximal tubules, but they appeared to be more numerous in Groups 3 and 4, suggesting a possible association to renal tubular cell tumor promotion (data not shown).

Fig. 2 shows a representative electron micrograph of a renal tubular cell tumor. As seen by electron microscopy, the tumor cells were arranged in tubular-like structures and had pleomorphic nuclei with quite prominent nucleoli. The cytoplasm was full of ribosomes and also had numerous small mitochondria. The cytoplasm also had some rough endoplasmic reticulum. Another interesting observation is that all of the tumors examined under electron microscopy had brush-border-like microvilli, but in inappropriate locations. In many of the cells, the microvilli were internalized. This had been described previously by the excellent electron-microscopic work of Hard and Butler (11) and Dees et al. (5). There was no apparent difference in ultrastructure between the renal tumor in the rat treated with DEN alone compared to the tumors promoted with nicotinamide.

DISCUSSION

The results of this study demonstrate that nicotinamide had renal tumor-promoting properties in rats initiated with DEN. Renal tubular cell tumors were induced by the end of 20 months in 59% of the kidneys of rats given a single i.p. injection of DEN 24 hr post-two-thirds partial hepatectomy and then put on 30 mm nicotinamide in the drinking water. The rats on the lower dose of nicotinamide had fewer renal tumors, but the incidence of tumors was still statistically significant from Group 1 (DEN alone).

No renal tumors were found in the rats treated with nicotinamide without DEN initiation (Group 2). The lack of direct carcinogen-
Nicotinamide Effect on DEN-Induced Renal Tumorigenesis

Nicotinamide effect on the development of renal tumors in rats treated with DMN (7, 18, 19, 27, 35, 48), streptozotocin (44), or EHEn (13, 14). N-(3-5-Dichlorophenyl)succinimide and citrinin induce interstitial nephritis and enhance DMN or streptozotocin renal tumorigenesis (19, 42, 44). DMN and streptozotocin induce both epithelial and mesenchymal tumors, but EHEn induced only renal tubular cell tumors (7, 12–14, 17–19, 22, 27, 36, 45, 48, 51).

Recently, it has been shown that sodium arsenite promoted renal tubular tumors induced by DEN (45). In that article, no mention was made about the toxic effect of sodium arsenite on the kidney. The 2 agents which have been suggested to promote renal tubular cell tumors were β-cyclodextrin and basic lead acetate (13, 14). Basic lead acetate was shown to induce renal tumors at high concentrations and has also been shown to cause degeneration of proximal convoluted tubules (4, 13, 50). Thus, the validity that basic lead acetate is a tumor promoter is questionable in view of the fact that basic lead acetate by itself is carcinogenic. β-Cyclodextrin was given for only 7 days, and renal tumors were observed at 32 weeks. Thus, the tumor-promoting effects of β-cyclodextrin was probably not reversible (14). An important characteristic of tumor promoters in general is their reversibility and, because of this, β-cyclodextrin does not qualify as a true tumor promoter. It also induced degeneration of proximal convoluted tubular cells and might have acted as a stimulus for DNA synthesis to “fix” the cancerous damage as has been postulated as the cause of increased liver tumors when carcinogens are given before and/or after a partial hepatectomy or carbon tetrachloride treatment (8, 33, 34).

In our model of inducing renal tumors, no mesenchymal tumors were found. Histologically, all tumors appeared the same. This differs from other reported protocols producing renal tumors. Most investigators report at least 2 distinct histological types: those with basophilic cytoplasm, the so-called “dark” cell tumor, and those with clear cytoplasm, the so-called “clear” cell tumor (5, 12–14, 17, 19, 43). Many other investigators have described the morphogenesis of renal tumor cell tumors (5, 6, 43). They appeared to be more numerous in the groups treated with nicotinamide, already demonstrated in mice (49), is also shown for the rats in our study.

There have been reports recently on the effects of various factors on the development of renal tumors in rats treated with nicotinamide (10). Hander and Dunn (10) reported that 1% nicotinamide in the dose of nicotinamide increased the number of kidney tumors to adenomas to carcinomas. We also observed clusters of the morphogenesis of renal tumor cell tumors (5, 12–14, 17, 19, 43). Many other investigators have described many other investigators have described nicotinamide and DEN (Groups 3 and 4), thus suggestive of a possible association with renal tumor promotion.

Nicotinamide has been shown to have opposing effects on carcino genesis (29, 36, 40, 41). In some systems, nicotinamide increased the incidence of pancreatic tumors but decreased the incidence of kidney tumors (36). In another study, a single high dose of nicotinamide increased the number of kidney tumors induced by DEN prenatally (41). It has been postulated that the effects of nicotinamide are due to increasing the NAD pools which are depleted by certain carcinogens (36, 40). Nicotinamide has also been shown to deplete the choline stores in the liver (10). Hander and Dunn (10) reported that 1% nicotinamide in the diet inhibited the growth of rats. Other investigators have reported that as low as 0.1% nicotinamide can inhibit the growth of rats (21). We confirmed their studies and showed that, with long-term nicotinamide feeding, the rats on 30 mw nicotinamide (0.33%) had a marked reduction in body weight.

As mentioned in the “Introduction,” partial hepatectomies were performed in the rats used in the study because our protocol was designed to investigate the possible role of nicotinamide in hepatic neoplasia. No significant differences in hepatoma number between the different groups were seen. There is no reason at this point to believe that the two-thirds partial hepatectomy was an essential feature of the protocol for the development of kidney tumors, though it is possible that the dose of DEN delivered to the kidney may be larger in the partially hepatectomized animal (7, 35). The role of other procedures such as unilateral nephrectomy will be investigated in future studies.

In conclusion, we have found that nicotinamide is a tumor promoter of renal tubular cell tumors which appears to be nontoxic by light microscopy and not directly carcinogenic to the kidney. Thus, this is the first renal tubular cell tumor promoter which appeared to have the same properties as the promoters described in the skin and the liver. Further studies need to be done to try to determine the mechanism of action of nicotinamide as a promoter of renal tubular neoplasia.

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References

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Fig. 1. Photomicrograph of a renal tubular cell tumor induced with DEN and promoted with nicotinamide. A, x 200; B, x 800.
Fig. 2. Electron micrograph of a renal tubular cell tumor induced with DEN and promoted with nicotinamide. × 4600.
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