Transitional Cell Hyperplasia in the Bladders of Dogs Fed DL-Tryptophan

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SUMMARY

Purebred beagle dogs have been fed a diet supplemented with DL-tryptophan, 6 g/day, a dose which increased their normal daily intake of this amino acid approximately 7-fold. Marked focal hyperplasia of the transitional epithelium of the bladder was produced, accompanied by lymphocytic and macrophagic infiltration of the submucosa. These effects were uniformly observed in dogs fed this dose for from 3.5 months to almost 7 years. No correlation was observed between the severity of the effect and the period of feeding. These results suggest a possible role of tryptophan as a cocarcinogen in the induction of bladder cancer.

INTRODUCTION

Although it has been well established that 2-naphthylamine and other aromatic amines produce bladder cancer in both man and dogs (5), it seems likely that exposure to these substances only partially explains the incidence of this disease in humans. Therefore, other causative agents must be sought. Many separate lines of experimental evidence in animals have indicated the possible involvement of the amino acid tryptophan in human bladder cancer. The 1st of these was a classic experiment performed 20 years ago by Dunning et al. (6). This experiment demonstrated that the administration to rats of DL-tryptophan combined with 2-acetylaminofluorene produced a high incidence of bladder tumors. No bladder cancer was produced from the administration of either DL-tryptophan or 2-acetylaminofluorene alone, although tumors of the liver and ear duct were produced by 2-acetylaminofluorene. This observation, coupled with the knowledge that tryptophan is excreted in the urine as a variety of metabolites, some of which are o-hydroxyamines, led to a continuing investigation of the role of tryptophan in the induction of human bladder cancer. The theory that o-hydroxyamines are the etiological agents in bladder cancer has been superseded by the belief that the N-hydroxy metabolites are the proximal carcinogens (12). However, as is sometimes the case, it is not unlikely that the original premise was correct for the wrong reasons.

Considerable research effort has gone into the investigation of tryptophan metabolite concentrations in bladder cancer patients as compared with normal controls (2, 10, 11). Most experiments have indicated the presence of significantly elevated levels of certain tryptophan metabolites in the urine of about one-half of the patients suffering from bladder cancer of unknown etiology (2, 14) but not in the urine of bladder cancer patients who suffered from industrial exposure (1). In addition, the carcinogenic action of the urinary metabolites of tryptophan has been tested by implantation in the bladders of mice. Significantly increased incidences of bladder tumors in mice were observed with 3-hydroxy-L-kynurenine, 3-hydroxyanthranilic acid (3, 4), 8-methyl ether of xanthurenic acid, xanthurenic acid, and 8-hydroxyquinaldic acid. In addition, other investigators (13) have shown that 3-hydroxyindoleacetic acid and methyltryptophan are leukemogenic in C57 mice, there is a report (8) that L-tryptophan is capable of enhancing the hepatocarcinogenesis produced by N-nitrosodiethylamine in rats, and the etiology of bladder cancer from smoking cigarettes has been ascribed to the elevation of urinary metabolites of tryptophan (7, 9).

For more specific evaluation of the tumorigenic potential of tryptophan, the experimental feeding of DL-tryptophan to dogs was initiated in August 1963. In 1968, after the administration of approximately 7 kg of DL-tryptophan to each dog, following negative cystoscopic examinations, 2 of these animals were sacrificed. When the unusual hyperplastic nature of the bladder mucosa was observed, an additional short-term experiment was initiated.

MATERIALS AND METHODS

Chronic Feeding Experiment. The dosage of DL-tryptophan administered to 4 purebred female beagle dogs was 6 g/day added to a basal diet of Purina dog meal. The dogs were fed approximately 300 g/day of the dry meal, to which water and the DL-tryptophan were added. The food was well mixed before it was offered to the dogs. Each dog ingested its total daily diet. It was calculated that the amount of tryptophan contained in the basal diet was about 1 g. Thus, the level of tryptophan consumed by the experimental dogs was increased approximately 7-fold by the supplemental feeding. Four female purebred beagle dogs were maintained on the basal diet (unsupplemented by tryptophan) as controls for approximately the same experimental period (7 years).

Subacute Experiment. Following the completion of the chronic feeding experiment, a short-term experiment that used the same feeding procedure was carried out. Four purebred beagle dogs were fed DL-tryptophan for 1.5, 3.5, 14, and 15.5 months, respectively.

Histopathology. At the end of the feeding period, the dogs were sacrificed by electrocution, after the bladders had been

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emptied by catheter. The bladders were removed and fixed in 10% formalin. Sections were cut and stained with hematoxylin and eosin.

RESULTS

Chronic Experiment. After 55 months of tryptophan administration to the original 4 beagle dogs, the urinary bladders were examined by cystoscope. No abnormalities were observed. Up to this point, each dog had consumed approximately 7 kg of supplemental DL-tryptophan. We decided to terminate the experiment and sacrifice the animals. However, when Dog 1 was sacrificed, the bladder presented a most unusual appearance (Fig. 1). The entire mucosa was darkened and covered with circular gray-white areas, 1 to 2 mm in diameter. DL-Tryptophan was fed to the remaining 3 dogs for an additional 5 months, at which time a 2nd dog was sacrificed. The bladder mucosa of Dog 2 was similar in appearance to that of Dog 1, although the light areas were somewhat less striking (Fig. 2).

Histological examination of the bladders of these dogs revealed hyperplasia of the transitional epithelium. In both dogs, the mucosal layer, in areas, was 10 to 12 transitional cells thick (Fig. 4), compared with a normal thickness of 3 to 4 cells, observed in the controls (Fig. 3). Edema of the stroma was noticeable. Small clusters of lymphocytes were frequently observed (Fig. 5) in the submucosa immediately below the basement membrane. It is possible that these nodular infiltrations of lymphocytes were responsible for the whitish areas observed grossly. However, no cysts or gland-like structures typical of cystitis cystica or cystitis glandularis were observed. The transitional cells frequently contained vesicles in the cytoplasm surrounding the nucleus which had a clear or slightly hemophilic cast. In Dog 1, large areas of the bladder were denuded of transitional cells. At this point, we decided to continue feeding DL-tryptophan to the remaining 2 dogs to determine whether additional pathological changes would develop. After total feeding times of 81 and 83 months, respectively, the remaining 2 dogs (Dogs 3 and 4) were sacrificed. The bladder mucosa of Dog 4 presented an appearance similar to that observed previously. However, the bladder of Dog 3 was almost normal in appearance.

Histological examination of the bladder of Dog 4 presented the same picture as did those of Dogs 1 and 2. Marked hyperplasia with lymphocytic infiltration and nodules of lymphocytes was observed. However, Dog 3, which was fed tryptophan for 81 months, showed only a slight, but still definite degree of hyperplasia. More distinctive was the vesiculation of the cytoplasm surrounding the nucleus. Thus, in 3 of the 4 dogs, marked hyperplasia and lymphocytic infiltration was observed. These changes are consistent with a picture of chronic irritation.

Subacute Experiment. The above-described chronic experiment with 4 female beagle dogs was designed to test for the production of bladder cancer by DL-tryptophan. Since bladder tumors may take as long as 6 or 7 years to develop, some of the dogs were fed for the prolonged period of almost 7 years. However, the observed effect, bladder hyperplasia, is generally a more short-term effect and probably would not require 7 years to develop. Therefore, a short-term experiment was carried out. No pathological changes were observed in the bladder of Dog 5, fed tryptophan for 1.5 months (Fig. 6). However, after 3.5 months of feeding, marked hyperplasia was evident in Dog 6 (Fig. 7). The presence of hemosiderin-containing macrophages was also noted. The 2 dogs fed tryptophan for 14 and 15.5 months were also markedly hyperplastic. In Dog 7, squamous metaplasia of the transitional epithelium was observed (Fig. 8), and 1 apparent hyperplastic papillary projection was observed microscopically (Fig. 9) that was not observed on gross examination. In these dogs, as in those previously described, additional evidence of chronic irritation was observed, especially lymphocytic and macrophagic infiltration. Hyperemia of the submucosa beneath the areas of hyperplasia was also observed. In most of the dogs the hyperplasia seemed to be localized, rather than spread evenly throughout the mucosal surface, and seemed to be correlated with light areas observable to a variable degree on gross examination.

DISCUSSION

Perhaps the most widely held theory of chemical carcinogenesis considers that it is at least a 2-stage process. The 1st stage (initiation) is considered to be a thus far undetected
change in a critical cell constituent. This change remains latent. Many believe that it may consist of an alteration of the structure of the DNA of the cell nucleus. Subsequent exposure to a promoter or cocarcinogen, generally an irritant substance, produces cellular multiplication, encouraging the expression of the initiative phenotypic change. The results of these experiments add further fuel to the concept that tryptophan may somehow be involved in the induction of bladder cancer in man and may delineate that possible role. In the beagle dog at least, D,L-tryptophan produces marked focal hyperplasia of the transitional epithelium. Lymphocytic and macrophagic infiltration occurs, which completes the picture of chronic irritation occurring in the mucosa and submucosa in response to the administration of this amino acid. It is perhaps not surprising that maximal production of hyperplasia was observed after 14 months of feeding (Table 1). Dog 3, fed for one of the longest periods of time, indeed showed less effect from the feeding than did Dog 5, fed for 1.5 months. This observation may be ascribed to individual variation in the concentration of the irritant metabolite, whatever it is, and to the apparent fact that the production of hyperplasia is a relatively short-term matter.

Although no bladder tumors were produced by the prolonged treatment of these dogs (approaching 7 years), the possibility cannot be excluded that tryptophan alone is capable of the induction of bladder tumors in humans. There are some indications that humans are considerably more susceptible to the effects of bladder carcinogens than are dogs. A minimum amount of 2-naphthylamine (5 mg/kg/day) is required to produce bladder tumors in the dog, and surely this must be a huge dose in comparison to the amount absorbed during accidental industrial exposure. In addition, the histopathological changes observed with tryptophan are similar to the early changes recently observed in dogs treated with 2-naphthylamine. On the other hand, it seems much more likely that the induction of hyperplasia and lymphocytic infiltration by 2-naphthylamine are reflections of the promoting action of this substance. 2-Naphthylamine is obviously a complete carcinogen, capable of both initiation and promotion.

Because of the prohibitive cost of the pure L isomer, until very recently all tryptophan feeding experiments have been carried out with the racemic mixture. Therefore, the possibility must be considered that the effects of experimental tryptophan administration are related to the D isomer rather than to the L isomer. This seems a little unlikely, since it is known that D-amino acids are frequently oxidatively decarboxylated to the keto acid, from which point they can either be reaminated to the L isomer or enter the normal pathways of L-tryptophan metabolism. How important this "isomerization" process is, quantitatively, in the dog is not known. Recently, L-tryptophan became available at a more reasonable price, and a feeding experiment designed to settle this point is under way.

One wonders about the toxicological significance of these observations from the point of view of human tryptophan ingestion. The daily intake of tryptophan in our dogs, due to the protein content of the basal diet, was estimated to be about 1 g. Therefore, the administration of 6 g represents about a 7-fold increase in the level of tryptophan ingested by these dogs. Most meats contain 1 to 2% of L-tryptophan. However, the minimum daily requirement for L-tryptophan is only about 200 mg/day for an adult. Therefore, it seems likely that the human daily requirement for tryptophan could be met on an animal protein intake much below the present average intake in the United States.

Additional investigations are necessary to discover the active hyperplasia-producing urinary metabolite, followed by a study of the occurrence of this metabolite and the factors influencing it in human urine.

REFERENCES

Fig. 1. Dog 1 was fed DL-tryptophan for 55 months. Gross appearance. Note darkened mucosa and sharply delineated white plaques covering surface.

Fig. 2. Dog 2 was fed DL-tryptophan for 60 months. Gross appearance. Similar to Dog 1, but plaques are somewhat less distinct.

Fig. 3. Normal mucosa from control dog. Transitional cell layer is 3 to 4 cells thick. × 70.

Fig. 4. Dog 2 was fed DL-tryptophan for 60 months. Mucosa is 10 to 12 transitional cells thick in some areas. × 70.

Fig. 5. Dog 1 was fed tryptophan for 55 months. Lymphocytic infiltration apparently is associated with hyperplasia. × 40.
Fig. 6. Dog 5 was fed DL-tryptophan for 1.5 months. Shown is normal mucosa, 3 to 4 transitional cells thick. X 220.

Fig. 7. Dog 6 was fed tryptophan for 3.5 months. Note marked hyperplasia and presence of hemosiderin-containing macrophages. X 220.

Fig. 8. Dog 7 was fed tryptophan for 15.5 months. Note squamous metaplasia. X 220.

Fig. 9. Dog 7. Hyperplastic papillary projection. X 110.
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