Study on Histopathology of Mouse Forestomach Neoplasia Inducd by Benzo(a)-pyrenc

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Abstract **Purpose** To explore the histopathological change during the development of mouse forestomach neoplasia inducd by benzo(a)-pyrenc B(a)P. **Methods** Mouse were killed after mouse were given benzo(a) pyrene (5mg/ml) per mouth twice per week for four weeks. The histopathology of forestomach was observed each time stage. **Results** Forestomach neoplasia of mouse was noticed from 16th week. Local hyperplasia of glandular organ, arrangement disorder, widely atypical hyperplasia of glandular organ, earlier forestomach cancer and aggressive forestomach cancer could be observed gradually. **Conclusion** Stage of latency of mouse forestomach neoplasia induced by B (a)P was about three months. The developing course was atrophic gastritis, atypical hyperplasia and forestomach cancer.

Key Words benzo(a)pyrene; forestomach neoplasia; atypical hyperplasia; forestomach cancer

There were many chemicals which could induce mouse forestomach neoplasia, such as benzo(a) pyrene (B(a)P), methyl-benzyl-nitrosamine, nitroso–sarcosine ethyl ester, etc. The method of B(a)P inducing mouse forestomach neoplasia had been used to check whether anticancer chemicals could inhibit or delay stomach carcinogenesis[1,2]. But up to now the development changes of mouse forestomach neoplasia histopathology induced by B (a)P had not been reported in the documents. Therefore, the logical changes of forestomach were observed during the development of forestomach neoplasia induced by B(a)P.

MATERIAL AND METHODS

Animals and group

Kunming species female mice provided by animals room of Harbin Pharmaceutical Group, body weight was 17-20g. Mouse were divided into 14 groups randomly, 15 mouse per group. Among of them, 7 groups were B(a)P groups and the other 7 groups were control groups.

Reagents

B(a)P was from Fluka corporation(purity 98%). Salad oil was from the market.

Methods

B (a)P was dissolved in salad oil, which concentration was 5 mg/ml. Mouse in B(a)P groups were given B(a)P per mouth according to 0.2 ml/20g bw, twice per week for four weeks. Mouse in the control groups were given salad oil based on above-mentioned method. Body weight of Mouse were weighed one time per week. One group of mouse of B (a)P and control groups were killed respectively in 5th week, afterward one group of B(a)P and control group mouses were killed respectively every four weeks. Mouse were fasted to diet, but were not refrained from water 24 hours before killed. Eyeballs were removed for blood. The whole stomach including part of esophagus were cut, and were split from pylorus along greater curvature of stomach. After cleaned with physiological saline, stomach were weighed and observed macroscopely, then fixed with 10% formalin, imbedded with paraffin, serial sections were made and dyed with hematoxylin-eosin.

RESULTS

Growth curve of mouse body weight and ratio of stomach and body weight

Mouse of B (a)P and control groups grew well each stage, and the average body weight of mouse got close each stage (Fig.1), which showed no

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significant difference. The ratio of stomach and body weight between two groups each stage also had no significant difference (Table 1).

Tumor incidence of mouse each stage

The forestomach neoplasia of mouse in B(a)P group could be observed in 16th week. With the prolongation of time, the tumor incidence in B(a)P group increased gradually, and the tumor incidence was 100% in 28th week (Table 2).

Histopathology observation

Macroscopic observation Gastric mucosal fold was obvious in control group each stage, there were no any macroscopic abnormal change (Fig.2). Gastric mucosa of B (a)P group was normal as that of the control in 4th and 8th week, in 12th week mouse gastric mucosal fold became shallow, even some of them nearly disappeared, and outer limit was very obvious contrasting to the circumambient normal mucosa. These phenomenon was more outstanding in 16th week. From 16th week, some mouse gastric mucosa appeared white protrution which was inequality of size. After 20th week, all mouse gastric mucosa appeared protrution, which was inequality of size and number, presented corpora mammillaria protruding to gastric cavity, but also protrution increased and augmented with the prolongation of time, maximum diameter was 4mm (Fig.3).

Microscopic observation Mouse gastric mucosa showed normal histology form in control group, glandular organ in the mucous layer was eumorphism and lined up in order, obvious abnormal hyperplasia was not observed (Fig.4). In B(a)P group, histology form of gastric mucosa was similar to the control group in 4th and 8th week. But part glandular organ which was near to lamina propria appeared glandular epithelium atrophia, abnormal shape and irregular arrangement, but did not diffuse toward superficial layer and infiltrate toward deep layer (Fig.5). Glandular organ was obviously abnormal hyperplasia in 16th week, and hyperplastic glandular organ concentrated and was close each other, there was a thimbleful of interstitial substance (Fig. 6); Some diffused toward superficial layer and manifested irregular or nodosity; Some infiltrated toward deep layer. From 20th and 24th week, cancer tissue had no obvious glandular arrangement, cancer cells were large and polymorphism, significant heteromorphism, nucleus became greater and strongly dyed (Fig.7). Besides above-mentioned manifestation, in 28th week cancer tissue infiltrated toward deep layer to muscular layer (Fig.8). Esophago showed no abnormal pathological change above on each time.

DISCUSSION

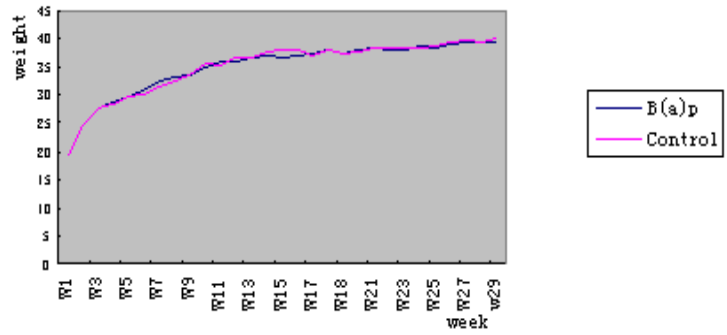
Benzo(a)pyrene was a kind of polycyclic aromatic hydrocarbon family chemicals which had carcinogenesis, and mass data suggested that B(a)P had carcinogenicity effect on human and varied an-

Table 1. The ratio of stomach and body weight of mouse

group	W4	W8	W12	W16	W20	W24	W28
B(a)P	0.91±0.14	0.99±0.23	0.95±0.26	0.86±0.12	0.85±0.12	0.90±0.16	0.94±0.13
Control	0.90±0.16	0.84±0.10	0.85±0.01	0.78±0.14	0.91±0.13	0.86±0.13	0.87±0.01

Table 2. The tumor incidence of mouse each stage in B(a)P group

week	number of mice	number of tumor of mice	tumor incidence(%)
4	15	0	0
8	15	0	0
12	14	0	0
16	12	4	33.3
20	15	11	73.3
24	13	12	92.3
28	12	12	100



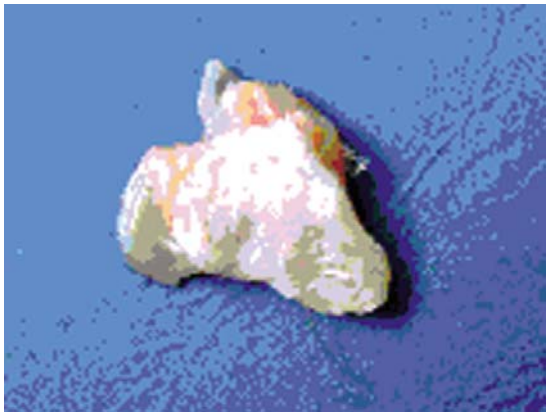


Fig. 2 Normal mouse forestomach

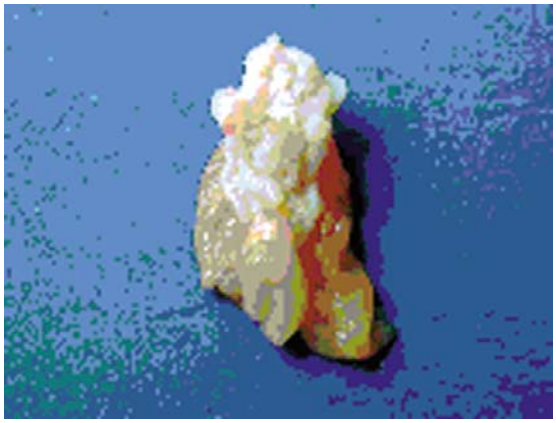


Fig. 3 Mosue forestomach neoplasia

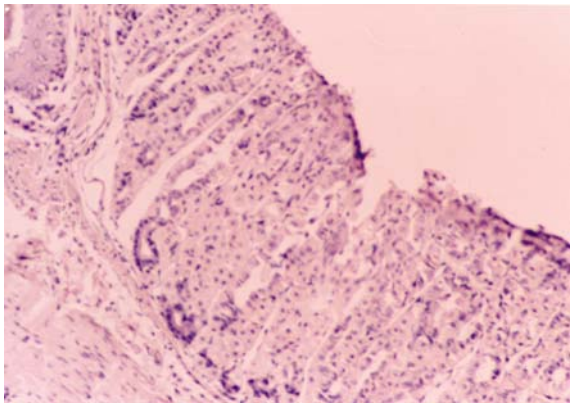


Fig. 4 Normal gastric mucosa (HE×40)

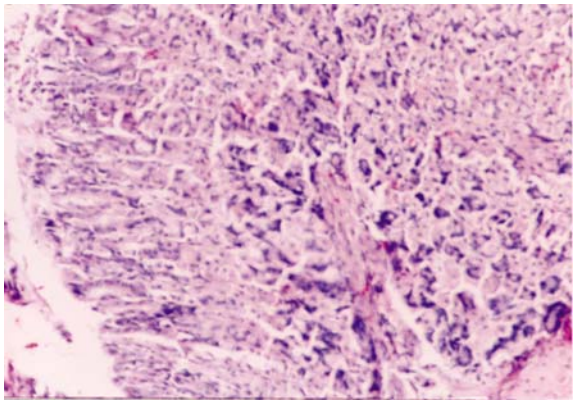


Fig. 5 Atrophy gastric mucosa (HE×100)

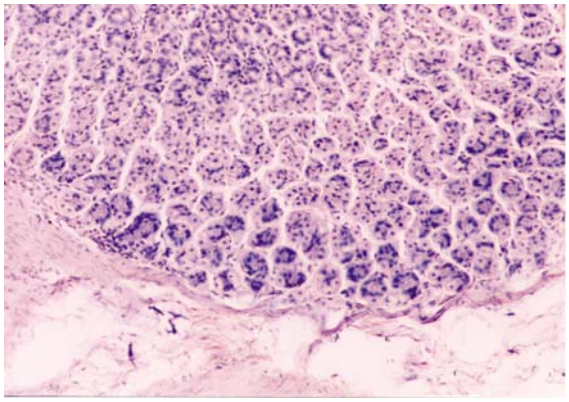


Fig. 6 Atypical hyperplasia of gastric mucosa (HE×100)

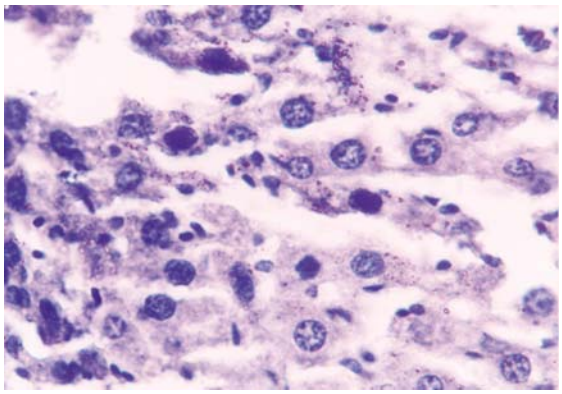


Fig. 7 Forestomach neoplasia (HE×400)

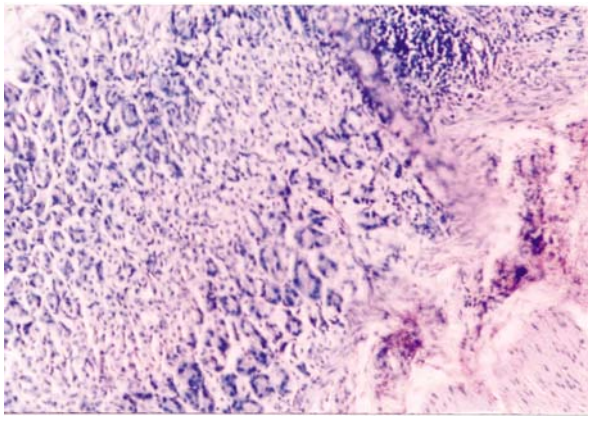


Fig.8 Progression forestomach neoplasia(HE×100)

imals. 2 mg B(a)P given one time per mouth could induce mouse forestomach neoplasia, and there was dose-response relationship. Given the feedstuff containing B(a)P mouse could be induced forestomach noeplasia. Moreover, with the prolongation of feeding time, lung cancer and leukemia also could be found, which also presented dose-response relationship.Rats could be induced esophago and forestomach papillary epithelioma if given 2.5 mg B(a)P per mouth everyday[3]. Through the catalysis of CYP1A1, B(a)P was metabolized and activated into dihydrodiol-epoxide-Benzo(a)pyrene(DEBP), then DEBP conjugated covalently with DNA to result in DNA injury, further made cell canceration. Now generally it was regarded as onset reason of a series of genetic injury induced by B(a)P that metabolism activatory B(a)P conjugated covalently with DNA.

Body weight of mouse in B (a)P and control groups presented parallel growth on the whole and had no difference, which suggested initial stage of canceration had no very effect on growth and development of mouse. Along with the advancement of tumor, whether the tendency of weight growth between two groups showed difference needed to prolong the breeding time to further observe. The ratio of stomach and body weight in B (a)P group exceeded that in control group each time stage, but there was difference in statistics. The reason might be that net body weight in B(a)P group was generally lower than that in control group, but absolute weight of stomach exceeded that in control group (no difference in statistics). So the results indicated the procedure which B (a)P induced mouse forestomach neoplasia was relatively slow, the stage of latency was about three months. Meanwhile, it also further validated that carcinogensis was a chronic, long-term process. Stomach cancer mainly originated from stem cell of cervical gastric glands, where regeneration and reparation ability of glandular epithelium was particularly actived, and it could differentiated into gastric epithelium and enteric epithelium, canceration usually began from that locus. These characters were also displayed in principle in this experiment. Firstly histology change of atrophic gastritis appeared, furthermore, inflammatory cells could be observed in various stages. After that, atypical hyperplasia of glandular organ came, following most time stage, but also different hyperplasia demonstrated that chemical factor resulted in different pathological changes of forestomach neoplasia in the experiment. Heavy atypical hyperplasia often appeared near cancer locus, some had migration relationship with cancer tissue. Intestinal metaplasia was not observed because forestomach neoplasia of mouse induced in this experiment located in cardiac part, but not pyloric part. Later period stomach showed the histopathology representation of cancer, that was to say, cancer cell had karyomegaly and strongly dyed, and heteromorphism was obvious. Therefore, this research successfully induced forestomach neoplasia of Kunming species mouse with B(a)P, atrophic gastritis, atypical hyperplasia and cancer were roughly the histopathological change procedures of mouse forestomach neoplasia induced by B(a)P. With the time prolongation, the cancer gradually developed. These were coincident to pro-cancer disease and precancerosis of stomach cancer found in the clinic and epidemiologic study [4].

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