Diethyl nitrosamine alone was not recommended for primary hepacelluar carcinoma model in rats

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Abstract: Objective: Liver cancer is the sixth most common cancer (782,451 new cases), the second cause of cancer related death (745,517 cases), and accounts for 9.1% of all cancers. The rat model is an important means to study the hepatocellular carcinoma (HCC). In our research, we evaluated the HCC rat model constructed by administration of diethyl nitrosamine (DEN). Methods: Ninety-six male adult Wistar rats (8 weeks, 180 g~220 g) were used to construct the HCC model. All rats were feed one year after they were injected intraperitoneally with normal saline (NS), 20 mg/kg/d DEN and 40 mg/kg/d DEN 8 weeks. Macroscopic examination and hematoxylin-eosin (HE) staining were used to evaluate the models. Conclusion: No rats formed the typical liver cancer lesion from liver cirrhosis. Thus, the administration of DEN alone is not recommended for HCC rat model.

Keywords: Diethyl nitrosamine (DEN), primary hepacelluar carcinoma (HCC), rats’ model

Introduction

Liver cancer is the sixth most common cancer (782, 451 new cases), the second cause of cancer related death (745,517 cases), and accounts for 9.1% of all cancers worldwide [1]. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers and is a major global health problem. The incidence of HCC increases progressively with advancing age in all populations, reaching a peak at 70 years. In Chinese and in black African populations, the mean age of patients with the tumor is appreciably younger [2].

Drug-induced liver cancer in rat is an important tool to study primary HCC. There are several drugs available to induce primary HCC in rats. Ethanol, carbon tetrachloride (CCl4), aflatoxin, nitrosamines, amino azo dyes, aromatic amine and so on are used to induce HCC in animal models. Diethyl nitrosamine (DEN) is a well-known potent hepatocarcinogenic agent present in tobacco smoke, water, cured and fried meals, cheddar cheese, agricultural chemicals, cosmetics, and pharmaceutical products [3-5].

DEN is known to induce damage in many enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models [6]. DEN-induced liver cancer in rats is still widely used as an important tool in the basic animal experiments. For example, Schiffer E. et al. [7] developed the HCC rats models by intraperitoneal injections of 50 mg/kg/week of DEN (total 12 weeks). In Su Bo et al’s study [8], rats were injected intraperitoneally with a dosage of 70 mg/kg of DEN once per week for 10 weeks. Deng Jie et al. [9] performed oral gavage daily with 5 mL/kg dose of 0.3% DEN solution for 12 weeks. Because several different doses and modes of administration have been tried in the past, it is difficult for a researcher to choose the dose and administration mode in the first attempt for developing DEN-induced HCC rat model. We hope our present study could provide appropriate guidance for such researchers.
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Material and methods

Animals and environment

All animal care and experimentation conformed to the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. Ninety-six male adult Wistar rats (8 weeks, 180 g to ~220 g) were bought from Vital River (Beijing, China). All rats were fed in the animal center of Shandong province hospital. They were maintained on a constant 12 h light/dark cycle under controlled temperature and humidity conditions. They had free access to tap water and standard rat pellets ad libitum. All experimental operations were in line with the Animal Ethics Committee. The rats were numbered and grouped the day after they were received. The experiments were formally conducted after 7 days adaptive feeding in the animal center. No rats died during the adaptive feeding period.

Chemicals and reagents

DEN (Sigma-Aldrich, N0756, St. Louis, MO) was of analytical grade. Normal saline (was from Baxter Shanghai, China).

Grouping and modeling

The rats were marked with 3% to ~5% picronitric acid. The labeling method is shown in Figure 1. Ninety-six male Wistar rats were randomized into groups by SAS software. The total time of intraperitoneal injection of NS and DEN was 8 weeks. We then continued to feed the rats for one year. There were four groups: A blank control group (9 rats), sham operation group (9 rats), 20 mg/kg/d DEN group (39 rats), and 40 mg/kg/d DEN group (39 rats).

We strictly observed the changes in the rats’ hair, behavior, and body weight.

Results

Hair and body weight

Though the tendency of 20 mg/kg/d DEN group was slightly slower than the blank control group, their body weight still increased slowly. (See Figure 2)

Analysis of rats that died accidentally

We dissected all rats that died during the experiment and did not find the formation of liver cancer. For example, Rat No. 92, which was assigned to the 40 mg/kg/d DEN group, died 134 days later. (Days were counted from the first day of DEN injection) We did not find the formation of liver cancer in this rat. The result is shown in Figure 3.

Analysis of rats at the end of the experiment

All rats were sacrificed at the end of the experiment. In our research, there were no rats with liver cancer.
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Discussion

Although there are many xenograph models, transgenic models, knock-out models, etc. [10], general rat models are still an important tool to study HCC, as they can render a better process of primary HCC’s natural development than mice or nude mice. Furthermore, rats can provide enough liver samples for related experiments. However, we do not recommend that researchers who lack time and sponsors should use DEN alone for the development of HCC rat model.

The weight of the 20 mg/kg/d DEN group rats increased more slowly than that of the blank control group, which indicated that DEN could impact the growth of rats. However, the sustainable growth in the first two months might predicate unsuccessful models in the short time.

We used large manpower, material, and financial resources for the experiment. E. Schiffer et al. [7] used 18 weeks to establish the HCC rat models induced by DEN. In our research, we spent more than one year on the development of the HCC rat models. This long modeling time would increase the uncontrollable accidents in the experiment. It would be more difficult to repeat the test in a shorter modeling time if the model failed. Repetitive process would increase both time and cost. Moreover, a typical model (from cirrhosis to cancer) of liver cancer was not formed in all the 96 rats, which proves that the modeling success rate is not high.

There are three ways to shorten the modeling time and improve the modeling success rate for the researchers who want to use DEN to develop typical cirrhosis. Several rats were found with HCC; however, the cancer was not formed because of cirrhosis. The result is shown in Figure 4.

Figure 3. Rat No. 92 with 40 mg/kg/d DEN (134 days). (A). Macroscopic examination of No. 92 rat’s liver shows no macronodular cirrhosis and HCC nodules. Photomicrographs of liver’s HE staining was shown at (B) low (×100) and (C) high (×400) magnifications.

Figure 4. DEN could induce the formation of HCC. Rats’ livers had no significant changes in blank control group and sham operation group. The liver showed an ulcer in the 20 mg/kg/d DEN group (red arrow). HCC was found in the 40 mg/kg/d DEN group (blue arrow). (A). Macroscopic examination (B) photomicrographs at ×100 magnification (C) Photomicrographs at ×400 magnification.
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op HCC rat models. The first approach is to use younger rats. Bo Su et al. [8] injected five-week-old male Sprague-Dawley rats intraperitoneally with a dose of 70 mg/kg of DEN once per week for 10 weeks. They then obtained HCC rats after 16 weeks. Eriko Taniai et al. [11] used 5-week-old F344/NS1c rats to obtain HCC models. The age of the rats might be an important factor in the development of a successful HCC model. Five-week-old rats were better than eight-week-old rats. The liver function of eight-week-old rats may be more perfect and mature than the five-week-old rats; hence, the former group could metabolize the drugs better than the latter group. Thus, DEN could not injure the liver easily in the eight-week-old rats. The second approach is to combine DEN with other drugs. Mahmoud A. Mansour et al. [12] used DEN and CCl4 to induce HCC rat models. They found occasional dysplastic nuclei six weeks later. Different drugs have different mechanisms to damage the liver, which will increase the modeling success rate. The third approach is to deliver a large dose once a week. For example, Bo Su et al. used a dose of 70 mL/kg/week of DEN. In our research, we use 20 mg/kg/d and 40 mg/kg/d to induce liver cancer. A large dose of DEN would exceed the liver’s ability to metabolize the drug. The excess amount of the drug that could not be metabolized at the first time will cause liver damage. Because the damage would be reduced over time, the drug should be administered repeatedly. One dose per week will give the liver adequate time to metabolize drugs and repair itself, which can reduce the mortality of rats modeled.

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Disclosure of conflict of interest

None.

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