

reported previously, the authors decided to study the inhibitory effect of A10 on hepatocarcinogenesis induced by AFB₁ in rats.

Materials and methods

Inbred male Fischer rats (Charles River Breeding Laboratories, Kingston, NY) were used in this experiment since these strains have been reported to be very sensitive to the hepatocarcinogenic effect of AFB₁ (24). The rats were housed two or three per cage in a temperature- and light-controlled environment with food (Formulab Chow No. 5008, Ralston Purina Co., St. Louis, MO) and water *ad libitum*. After 3 weeks of acclimatization, they were weighed, randomized, and divided into three groups (26 animals in each group): Group 1 = DMSO control; Group 2 = AFB₁ control; and Group 3 = test group. A control group administered only saline was not deemed necessary since dimethyl sulfoxide (DMSO) at doses used in this experiment was not shown to produce any change in body weight or liver morphology (25). The control groups were continued on stock pellets while the test group was switched over to food containing 1% of A10 (w/w) prepared from the same batch of food used for the control groups. This food regimen was continued for the entire period of the experiment. One percent of A₁₀ was selected based on other studies, which indicated that this is an optimal concentration (26).

Purified crystalline AFB₁ was obtained from Sigma Chemical Co., St Louis, MO. AFB₁ dosing for Groups 2 and 3 was started on the 8th day after A₁₀ feeding was initiated for the test group. AFB₁ was administered by gavage at the dose of 25 µg/day, 5 days weekly for 8 consecutive weeks (total dose = 1 mg/rat) (27, 28). Solutions were prepared freshly before each administration by dissolving AFB₁ in spectral grade of dimethyl sulfoxide (25 µg of AFB₁ to 50 µl of DMSO). Each dose was made up to 1 ml with distilled water. The

DMSO controls were given the vehicle. AFB₁ dosing was carried out in an alternating sequence between Groups 2 and 3, which was a measure taken to avoid any disparity between the groups in the time interval from preparation of solution to administration. Any suspected error while dosing, due to excessive struggle of animals, was taken care of by excluding that animal from the experiment.

Animals were carefully observed and weighed weekly. No special measure was taken to calculate the exact amount of food consumed by each group because body weight is shown to be a very sensitive indicator of caloric influence on tumourigenesis (29). Terminally ill rats were sacrificed and necropsied. Any animal that died during the experiment was also autopsied.

The experiment was terminated at 66 weeks from the first dosing of AFB₁. At necropsy, a detailed examination was made and the tumour incidence in each system was assessed. All the livers were weighed and the ratio of liver weight to body weight was calculated. The tumour incidence in liver was estimated depending on the size of the lesion (explained in the footnotes of Table II). Development of nodules and massive lesions made quantitation of foci unreliable. Hence, tumours measuring over 1 mm only were considered for incidence comparison between the two groups. Histological examinations were made on paraffin sections of tissues fixed in buffered formalin and stained with haematoxylin and eosin. The lesions were diagnosed according to the criteria of the Institute for Laboratory Animal Resources monograph on histologic typing of liver tumours (30).

The experiment was essentially designed based on other studies (31–33), to focus on the difference in incidence and morphology of liver tumours between the control and test groups. Further, A10 was not expected to produce any liver toxicity based on the extensive toxicology studies (18, 23, 34). Hence, we did not undertake the study of