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Humic Acid-Induced Testicular Morphological Changes in Rats

F.-J. Lu,[1] T.-C. Lee,[1] V. F. Pang,[2] T.-S. Huang[3]

[1]Department of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China
[2]Department of Veterinary Medicine, College of Agriculture, National Taiwan University, Taipei, Taiwan, Republic of China
[3]Department of Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China

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Humic acid is a fluorescent, deep brown organic acid which is easily found in various waters (such as wells, lakes, and oceans), humus and sediments (Flaig 1966). The molecular weight of humic acid can range from a couple of thousands to over tens of thousand (Kasparrov et al. 1981) because of forming polymers of phenolic acids. Most of its functional groups are phenolic, hydroxyl, and carbonyl groups (Stevenson 1982), enabling humic acid to bind with metals, especially arsenic (Lu et al. 1991).

Epidemiological studies have shown that there is a high correlation between humic acid and Blackfoot disease, a peripheral vascular disease prevailing along the southwest coast of Taiwan (Lu et al. 1991), and Kashin-Beck disease, a chronic osteoarthritis disease prevailing in the Mainland China (Wang et al. 1991). Humic acid can cause a variety of functional alterations such as shortening plasma prothrombin time and inhibiting plasmin activity (Lu and Lee 1992), inducing endothelin production (Chiu et al. 1993), enhancing the secretion of plasminogen activator inhibitor (Chiu et al. 199 1), enhancing the tissue factor expression and coagulant properties of cultured endothelial cells (Yang et al. 1994a), inhibiting protein C activity (Yang et al. 1994b), inducing blackening of tail and feet of mice (Lu 1990), inhibiting hepatic thyroxine 5'-monodeiodinase activity and affecting thyroidal function (Huang et al. 1993; 1994). Phthalic acid is one of the chemical degradation products of humic acid (Schnitzer et al. 1973), and it can induce testicular atrophy in the experimental rats (Cater et al. 1977; Creasy 1983). We report in this paper that humic acid has the same ability to induce testicular morphological changes in rats, and this ability seems to be enhanced by arsenic.

Correspondence to: F.-J. Lu

MATERIALS AND METHODS

Thirty grams of humic acid obtained from Aldrich Chemical Co., Milwaukee Wi, USA. were dissolved in 2 L of 0.1 N NaOH and centrifuged for 30 minutes at 13,500 xg to remove the sediments. The solution was then acidified with 6N HCl to make the pH value drop to 2.0 in order to precipitate the purer humic acid, and centrifuged for 30 minutes again at 13,500 xg. The procedure was repeated twice to get the purest humic acid. Later, the purified humic acid was then dried to a powder using a reducing-pressure evaporator. Double deionized water was then added to get the desired concentration (90 mg/ml) and the pH value of the solution was adjusted to 7.0. As_2O_3 was bought from Merk Chemical Co., Darmstadt, Germany, and no other preparation was required. As_2O_3 was dissolved in pH 11.0 double deionized water and then adjusted to the concentration of 6 mg/ml, pH 7.0. The solution of humic acid plus As_2O_3 contained 90 mg/ml of humic acid and 1 mg/ml of As_2O_3. All solutions were sterilized by passing through 0.8 μm cellulose acetate filter (Nalge Co., Rochester, New York, USA).

Twenty-seven Wistar male rats, weighing 220-270 g, obtained from the Animal Center at National Taiwan University were divided into four groups, 6 in the control group and 7 in each of the treated groups. The first treated group (HA group) was dosed with 90 mg/kg body weight of humic acid; the second group (As group) and the third group (HA+As group) were dosed with 6 mg/kg body weight of As_20_3 and 90 mg/kg body weight of humic acid plus 1 mg/kg body weight of As_20_3, respectively. The control group was given an equal volume of normal saline. All of the treatments were given by intraperitoneal injection every 6 days continuously for 168 days. No more than 3 rats were housed in each cage. Cages were changed periodically, and rats were allowed to eat and drink ad. libitum. The room temperature was controlled at 20 ℃. All rats were fasted for 24 hours and weighed before decapitation 2 days after the final treatment. Their testes were removed, weighed, fixed in neutral buffered formalin, and processed for light microscopic examination. Testicular sections at 5 μm thickness were stained routinely with hematoxylin and eosin. Special stains, including Berlin blue and AFIP lipofuscin, were done in selected sections for the detection of iron and lipofuscin, respectively.

RESULTS AND DISCUSSION

Grossly and microscopically, the testes of both control and As groups were normal (Fig. 1,2,3). However, the testes of 2/7 rats in the HA group and 3/7 rats in the HA+As group showed marked atrophy and light to dark brown

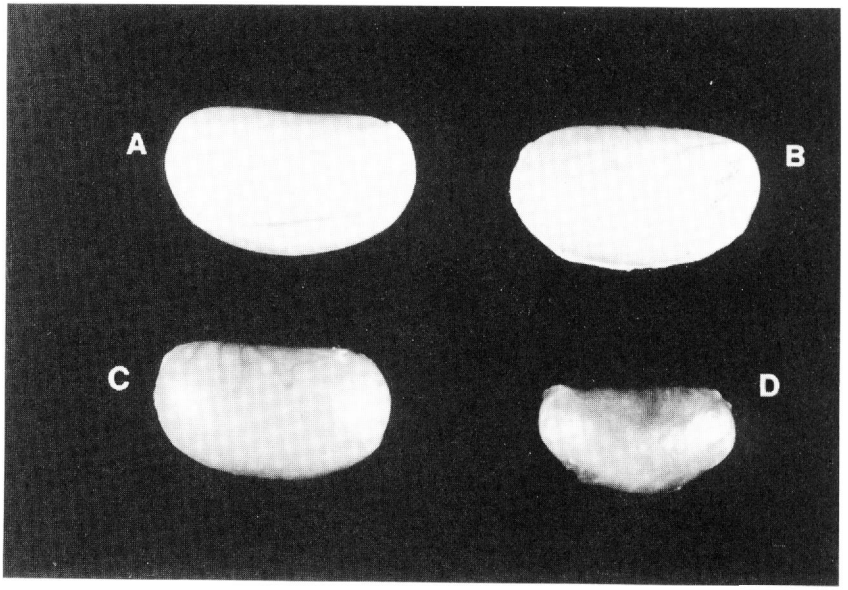

Figure 1. Gross appearance of rat testes.
(A): Control group: normal.
(B): As group: normal.
(C): HA group: mild atrophy and light brown discoloration.
(D): HA+As group: marked atrophy and dark brown discoloration.

discoloration grossly (Fig. 1). The mean testicular weights of the control, As, HA, and HA+As groups was 1.73, 1.70, 1.60, and 1.39 g, respectively. If only those grossly affected rats were included, the values dropped to 1.25 and 1.05 g for the HA and HA+As groups, respectively. Microscopically, the HA and HA+As groups showed capsulitis (Fig. 2), degeneration and atrophy of seminiferous tubules (Fig. 4,5), and interstitial cell hyperplasia (Fig. 6) in the testes of 6/7 and 6/7, 2/7 and 3/7, and 2/7 and 3/7 rats, respectively. The capsulitis was characterized by thickening of the tunics albuginea by fibrosis with infiltration of various numbers of macrophages and lymphocytes (Fig. 2). For those rats with grossly atrophied testicles, the percentages of seminiferous tubules injured were 20 and 100% for the HA group and 30, 80, and 100% for the HA+As group. The injured tubules were small and contained only some Sertoli cells and spermatogonia with no spermatids (Fig. 4,5). In addition, there was an absolute increase in the cellularity in the interstitial region; some of the cells also contained brown pigments. In those grossly unaffected testes of HA and HA+As groups, similar pigment-ladened cells, but less in the number, were also present in the interstitium. The cells containing brown pigments were assumed to be macrophages since similar pigment accumulation was also present in the

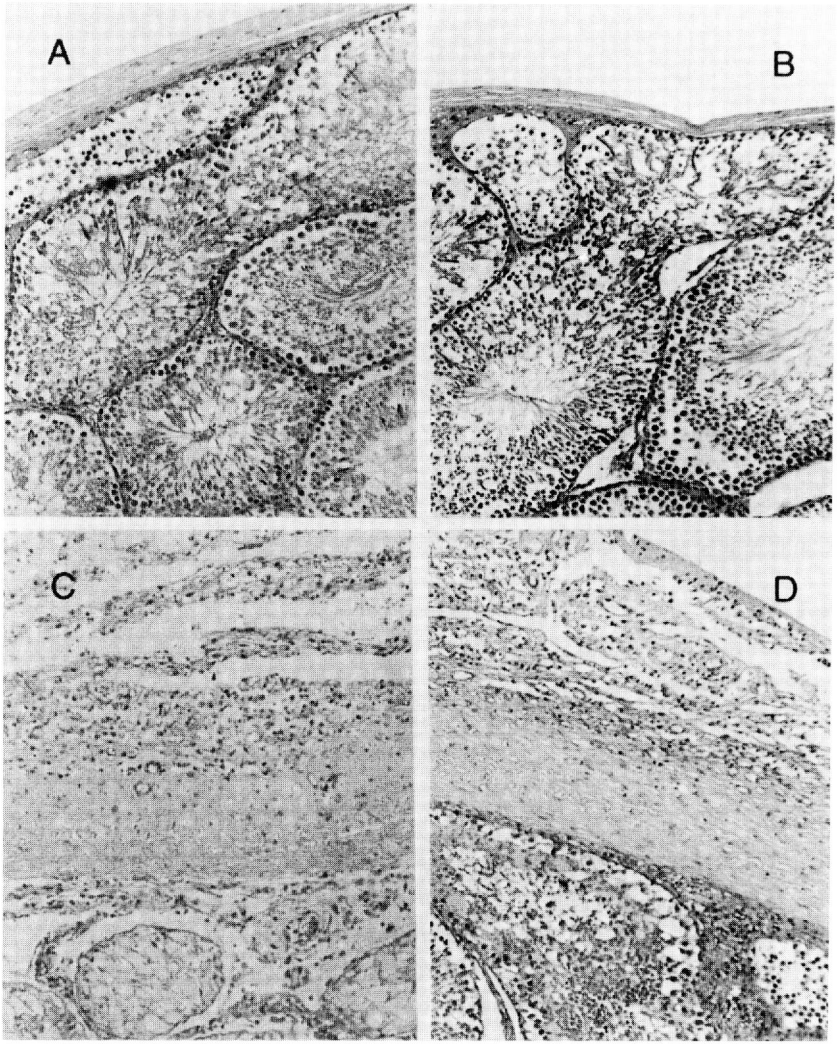

Figure 2. Testicular capsulitis characterized by microphage infiltration and thickening of tunics albuginea due to fibrosis (C,D).
(A) Control group.
(B) As group.
(C) HA group.
(D) HA+As group. H.E. stain. x1OO.

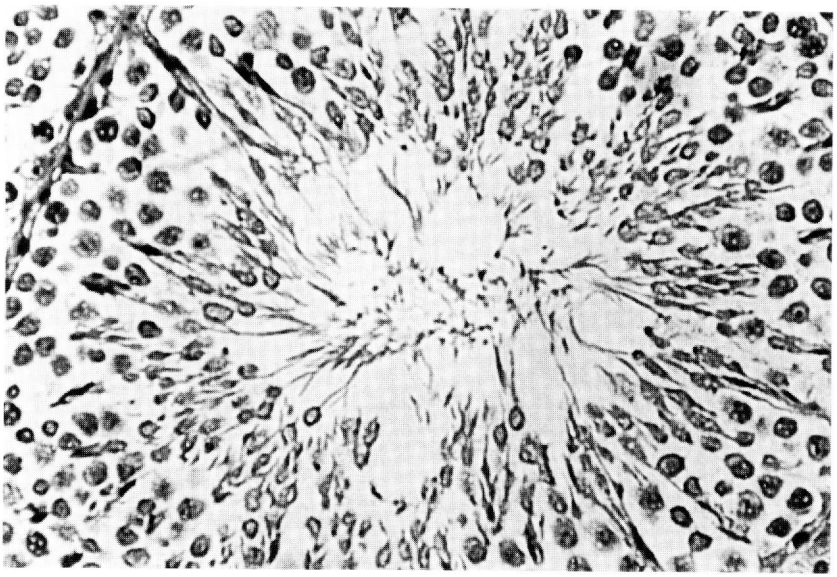

Figure 3. Testis from a rat of control group. Normal structure of a seminiferous tubule. H.E. stain. x400.

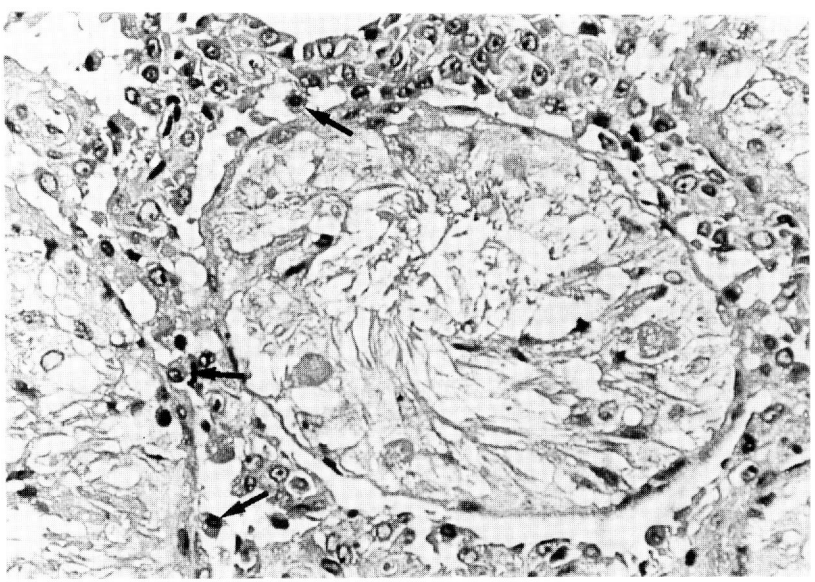

Figure4. Testis from a rat of HAgroup. Atrophy ofseminiferous tubules with loss of spermatogenesis and increase in interstitial cellularity, some of the cells containing pigments(arrows). H.E. stain. x 400.

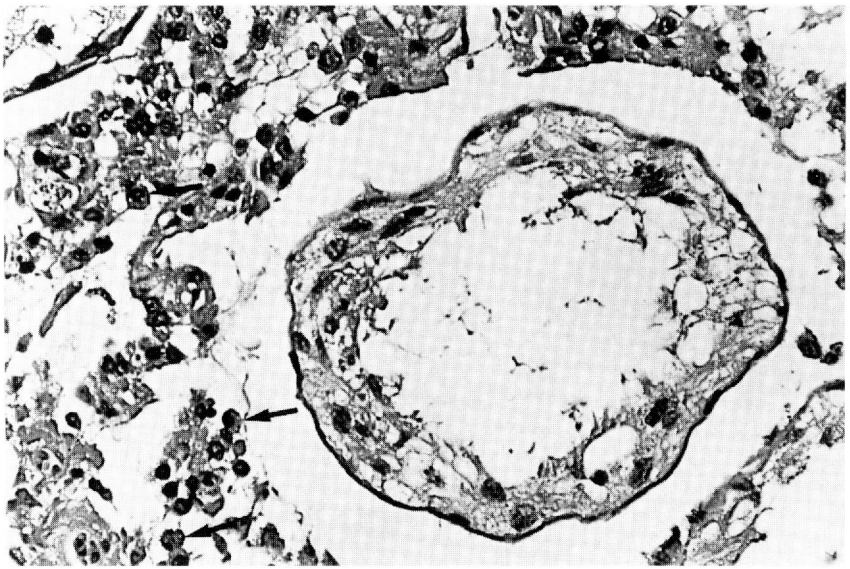

Figure 5. Testis from a rat of HA+As group. Atrophy of seminiferous tubules with loss of spermatogenesis and increase in interstitial cellularity, some of cells containing pigments (arrows).

Kupffer cells of the liver in the same animals (data not shown). It has been reported that Leydig cell hyperplasia is commonly seen in atrophied testicles (Wright et al. 1982). Therefore, the increased cellularity in the interstitium could be a result of hyperplasia of Leydig cells and /or infiltration of brown pigment-ladened macrophages. It is believed that the accumulation of brown pigment-ladened macrophages was the cause of testicular brown discoloration in the present study. The actual constituent of the brown pigments is still uncertain. They were partially positive for the AFIP lipofuscin stain but negative for the Berlin blue iron stain. Since humic acids are also brown in color, whether the brown pigments seen in the testis and other organs are a mixture of lipofuscin and humic acids remains to be answered. According to the incidence rate and severity of atrophy and brown discoloration, it seems that As_2O_3 has the ability to enhance the toxicity of humic acid, but As_2O_3 itself does not induce testicular atrophy in the rat.

The actual mechanism(s) of testicular atrophy caused by humic acid is unclear. Microscopically, the humic acid-induced testicular changes were similar to those caused by phthalate esters which is related to disrupted zinc metabolism (Cater et al. 1977; Creasy et al. 1983). Interestingly, phthalate esters are also components of humic acid (Matsuda and Schnitzer 1971;

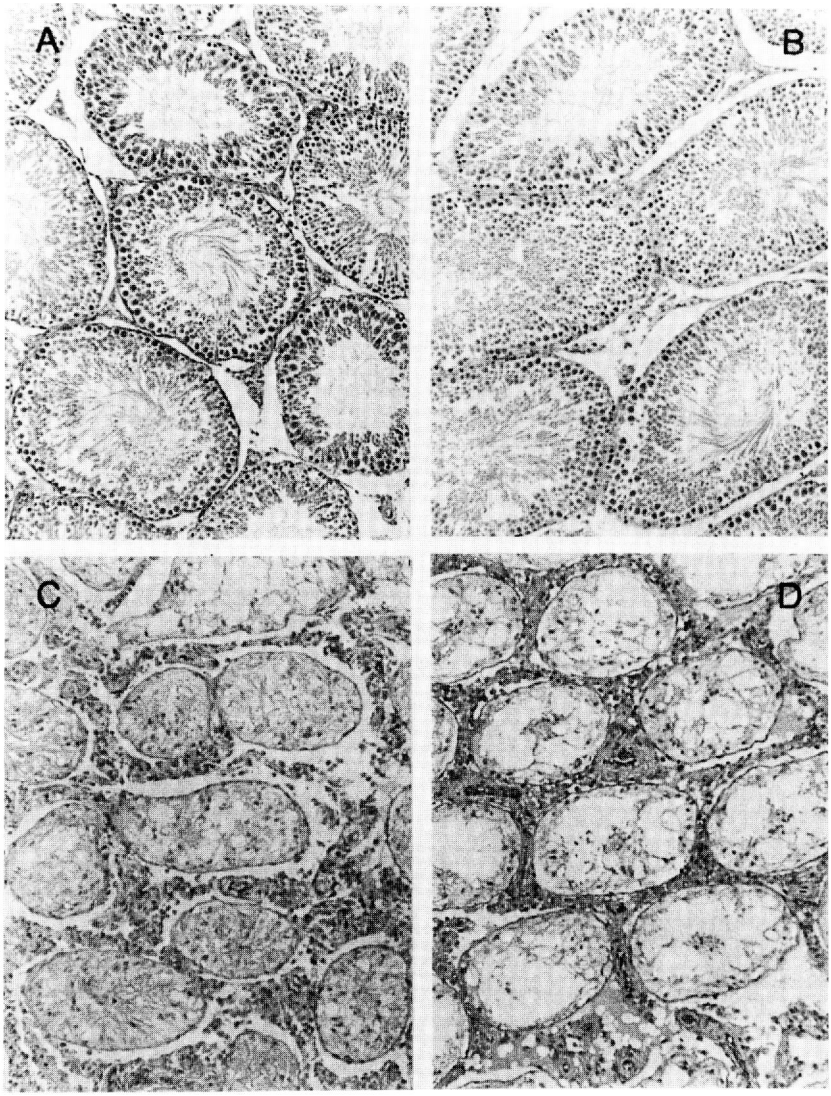

Figure 6. Hyperplasia of interstitial cells, atrophy and degeneration of seminiferous tubules (C,D).
(A) Control group.
(B) As group.
(C) HA group.
(D) HA+As group. H.A. stain. x1OO.

Schnitzer and De Serra 1973) and are widely distributed in the environment. Moreover, humic acids are chelators of various metal ions (Piccolo and Stevenson 198 1; Lu et al. 1988). Our previous studies have shown that humic acids can cause endothelial damage and endothelin production in tissue cultures (Chiu et al. 1993) and shortening of human prothrombin time (Lu et al. 1990). Additionally, we have also seen that humic acids can increase peroxisomes in the liver (unpublished data) and lipofuscin is a product of lipid perxoidation. Thus, changes in zinc metabolism, disturbed blood circulation, and/or enhanced lipid peroxidation may have played a role in humic acid-induced testicular injury.

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