Tr. J. of Medical Sciences
28 (1998) 369-374

Bilge ONARLIOĞLU[1]
Yasemin GÜNAY[1]
İsmihan GÖZE[2]
Emel KOPTAGEL[1]

Renal Ultrastructural Alterations Following Administration of Diethylstilbestrol

Received: January 8, 1997

Department of [1]Histology-Embryology,
[2]Medical Biology and Genetic,
Faculty of Medicine, Cumhuriyet University,
Sivas-Turkey

Abstract: Diethylstilbestrol (DES), nonsteroid in structure, is an artificial estrogen derivative and is used as an anabolic agent. It is known that diethylstilbestrol causes genetic disorders and toxic effects leading up to carcinoma even in amounts below its residual levels.

For this purpose, a dose of 60µm/kg/day of DES dissolved in 0,1 ml corn oil has been administered to rats by oral gavage for varying periods of 20, 50 and 100 days periods. Rat renal tissue samples, following routine electron microscopical procedure, have been examined by transmission electron microscopy.

Following diethylstilbestrol administration, glomerular capilleries seemed dilated along with occasional basement membrane thickenings and degenerations in slit membrane. In addition, irregular endothelial cell fenestrae were observed. Distal tubular structure remained intact but in the proximal tubuli, cells with picnotic nuclei and dilated intracytoplasmic foldings were present. These histological alterations increased parallel to the duration of the dosage period.

These findings suggest that the diethylstilbestrol usage may cause irreversible structural changes in rat renal tissue.

Key Words: Diethylstilbestrol, kidney, ultrastructure, rat.

Introduction

The diphenylethylene compound diethylstilbestrol (DES), a synthetic estrogen derivative displaying similarities to natural estrogens, is a human and a rodent carcinogen (1,2).

Although the effect mechanisms of DES known to cause different toxic effects still remain to be enlightened definitely, it is known that it has a slow and different metabolism when compared with that of natural estrogens (3,4).

Previous studies on the effects of DES in the prenatal development suggested decreased serum progesterone and total estrogen levels in different species (5). DES plays a depressive role in uterinal contractions and separation of the placenta during labour. This prenatal lethal action causes a definitive reduction in the number of offsprings (5,6).

In investigations made with DES during postnatal periods, its effects on rodents have been investigated. It has been reported that it causes uterus, vagina, cervix, ovarium and lymphoid tumors in mice (7), vagina, testes, kidney tumors in hamsters (8,9,10) and liver, vagina, mammary tumors in rats (3,11,12).

It has also been suggested that it caused genetic disorders besides carcinogenic effect in the same species and marked physiological and biochemical alterations, particularly in the renal tissues of rats without causing cancer (8,11,13).

Diethylstilbestrol has particularly been used as an anabolic agent in domestic animals but its usage has been prohibited in many countries following evidence that even amounts below residual levels lead to toxic effects and genetic disorders. Despite this, there are unfortunately no preventive measures taken in this direction in our country for domestic production (4).

In this study, it has been attempted to determine ultrastructural alterations likely to develop parallel to biochemical and physiological effects caused by DES on the rat kidney excluding cancer formation.

Materials and Method

Animals: Thirty-two male Wistar Albino rats,

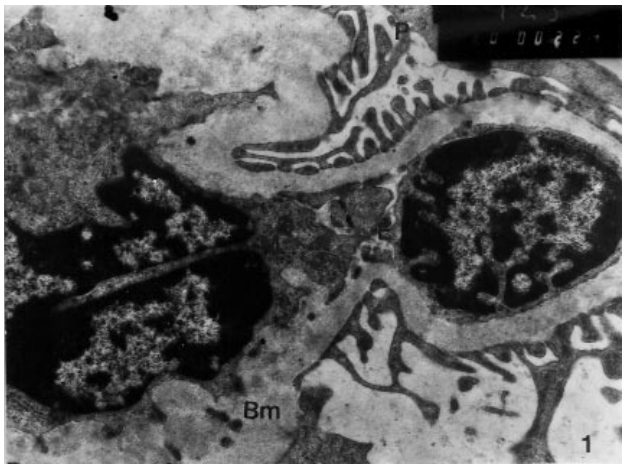

Figure 1. The glomerular structure in the control group. Endothelial cell (E), mesengial cell (M), podocyte (P) and the basement membrane (Bm). X 10.000.

weighing 200-250g were divided into 4 groups, each of which consisted of 8 animals. All animals used in the present study were obtained from the Experimental Animal Laboratory, Cumhuriyet University, SIVAS. They were bred and fed under standard laboratory conditions.

Experimental Procedure: The experimental group consisted of 24 rats separated into three groups DES (Sigma, Chemical Co. St. Louis, USA) at a dose of (60µg/kg/day) (12) dissolved in 0.1 ml corn oil was administered to the first experimental group (8 rats) for 20 days, to the second experimental group (8 rats) for 50 days and to the third experimental group (8 rats) for 100 days by oral gavage.

The control group rats (8 rats) were also administered 0.1 ml corn oil without DES by oral gavage.

Electron Microscopy: Tissues were fixed in 5% glutaraldehyde (pH:7.4) in phosphate buffer and further fixed in 1% osmium tetraoxide in phosphate buffer. Following fixation, tissues were dehydrated in increasing concentrations of ethanol, then embedded in Araldyte-CY 212 (Spy-Chem, Structure Probe INC, USA). Semi-thin and ultrathin sections were obtained using LKB IV ultratome (Bromma, Sweden). Ultrathin sections were stained by uranyl acetate saturated in 70% ethanol and Reynold's lead citrate (14). Ultrathin tissue sections were evaluated by JEOL-1200 EX-11 (Japan) transmission electron microscope.

Results

The Control Group

In the examination of samples obtained from the kidneys of rats belonging to the control group, endothelial cells in the glomerular capillaries, basement membrane, slit membrane formed by podocytes on it and a mesengial cellular structure having indented nucleus were observed (Fig.1).

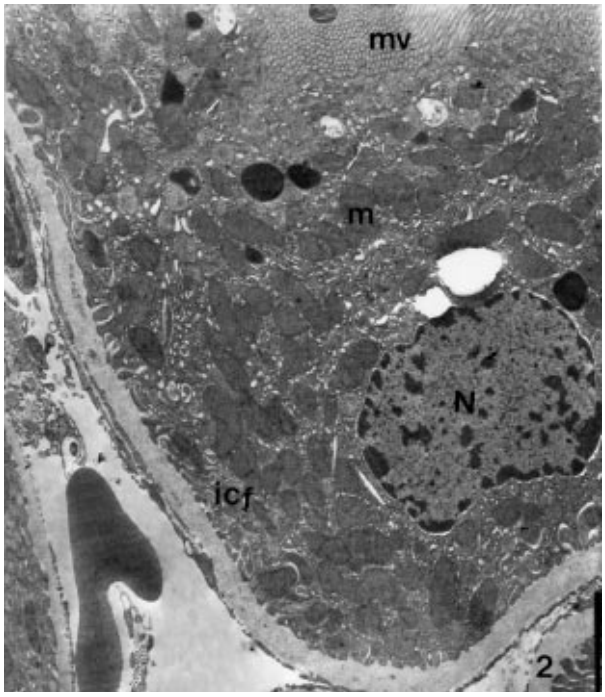

Figure 2. The proximal tubular cells with their microvilli (mv) and well-developed intracytoplasmic foldings (icf) in the control group. Nucleus (N) and mitochondria (m). X 5.000

As for the investigations made in the tubular area of the cortex, the proximal tubulus lined with cells characterized with their microvilli and well-developed basal foldings were observed (Fig.2).

The Experimental Groups

In the examination of the renal tissue belonging to Group I administered 60µg/kg/day DES dose for 20 days, it has been observed that the basement membrane in dilated glomerular capillaries thickened from place to place, developing into ondulation and the urinary space was closed up in many areas (Fig.3).

In the tubular area of the cortex, intracytoplasmic folding dilatations, electron-dense appearance in the mitochondria and thickenings in the basement membrane of the proximal tubulus cells attracted attention (Figs.4A-4B).

In the renal tissue of Group II, administered the similar DES dose for 50 days, the glomerular capillaries appeared to be dilated and hyperemic, the podocytes

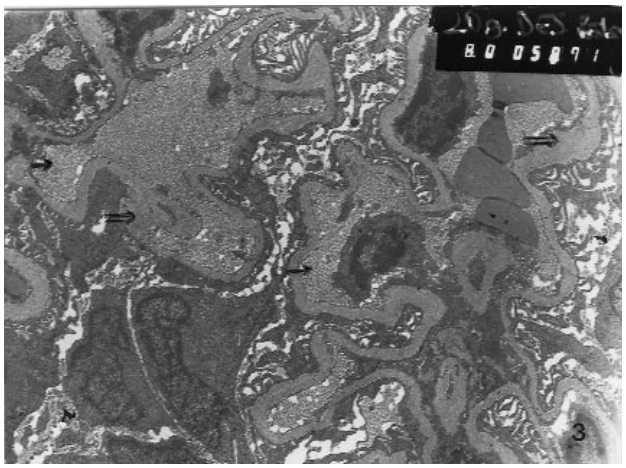

Figure 3. The glomerular structure belonging to Group I. In this electron micrograph, dilatation in glomerular capillaries (→), thickening and ondulation in the basement membrane (⇒) could be seen. X 8000

were exfoliated in many areas, the slit membrane structure deteriorated and the basement membrane becoming ondulated from place to place thickened (Fig.5).

In the tubular area, cells with picnotic nuclei in the proximal tubulus structure were encountered and dilatation occurred in the intracytoplasmic foldings of the cells observed to be rich in lysosomal structure (Figs.6A-6B).

In the tissue samples obtained from Group III, administered DES for 100 days, dilatation and hyperemia seemed to increase in the glomerular capillaries, occasional picnotic nuclei in the endothelial cells were encountered and the fenestrae of the cytoplasm could not be traced up. Ondulation, thickening and irregularities in the homogenous appearance in the basement membrane were noted. The podocytes were exfoliated in many areas and the slit membrane structure was deteriorated (Fig.7).

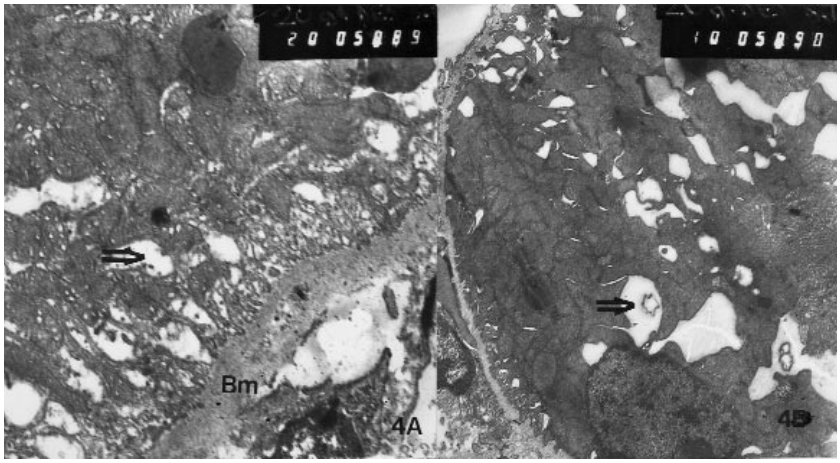

Figure 4A-4B. The tubular structure following DES administration for 20 days, thickenings in the basement membrane (Bm) and dilatations (⇒) in the intracytoplasmic foldings could be observed. 4A:X 20.000 4B:X 10.000

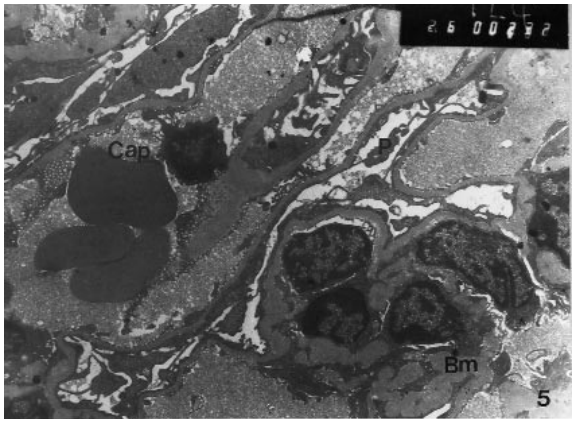

Figure 5. The glomerular capillaries (Cap) appeared to be dilated and hyperemic, podocytes (P) were exfoliated and the basement membrane (Bm) became ondulated and thickened in the renal tissue of Group II . X 2600

In the tubular area, particularly in the proximal tubuli, it was observed that the basement membranes of tubular cells rich in electron-dense mitochondria thickened and the intracytoplasmic foldings were dilated (Figs.8A-8B).

Discussion

Diethylstilbestrol defined as the main representative of artificial estrogens has a different and slow metabolism compared to that of natural and semisynthetic estrogens. In investigations on animals administered 4' hydroxypropiohenone developing as a result of metabolic activation of DES was held responsible for carcinogenous effect and the same metabolite was also observed in human beings (4).

As a result of administration of DES, the carcinogenic action of which is known to be its major effect, it has

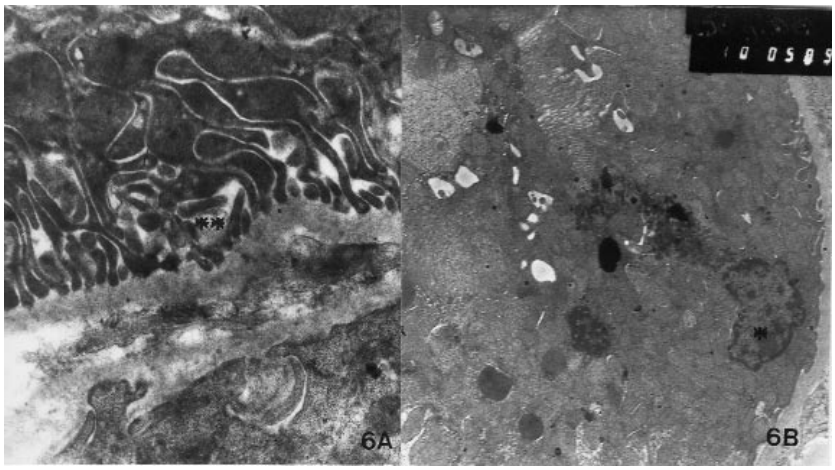

Figure 6A-6B. The proximal tubular cells with picnotic nuclei (*) and dilated intracytoplasmic foldings (**) could be observed in Group II. 6A: X16.000 6B: X10.000

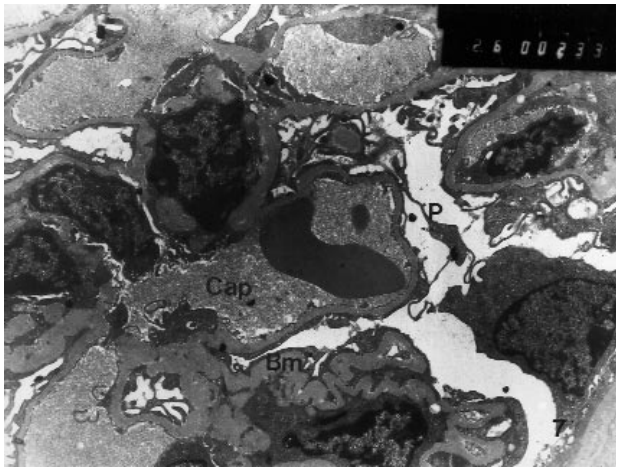

Figure 7. Electron micrograph of the glomerulus of Group III showing dilatation in the capillaries (Cap), degeneration in the podocytes (P) and thickening along with ondulation in the basement membrane (Bm). X2600

been observed that vagina, cervix, epididymis, testes, liver and kidney tumors developed in hamsters (8-10), and liver, vagina, mammary tumors in rats (3,11,12). Liver, vagina, uterus tumors were induced in mice (7).

As for DES administered to rats in the prenatal periods, it displayed a perinatal lethal action and a definitive reduction in the number of rat offsprings (2,5,6).

As a result of DES administration to rats in the postnatal period, it has been suggested that it also causes toxic actions on the renal tissue without displaying a carcinogenic effect (15,16).

In an investigation made, a morphological enlargement characterized by increases in the number of cells, the neck and tissue-fluid retention was determined in the renal tissues of rats administered DES (17).

In an other investigation made by Kinne and Kinne (16), it has been demonstrated that DES did not completely inhibit the Mg-ATPase activation of the proximal tubular cell brush border, but reduced it .

As for Katayama and Lee (1985), they carried out their investigations using natural and synthetic estrogens and demonstrated that DES particularly increased PGE_2 secretion in female rats and there was a parallel increase in Na excretion and tissue-fluid retention (15).

Although DES does not bring about structural alterations due to carcinogenic action on the rat renal tissue, it has been demonstrated that catecholes of the catechole cycle formed for the carcinogenic activation of DES as a result of testing all liver and kidney microsomes that had developed. It has consequently been argued that DES may assume a procarcinogenous role (8,18).

In this investigation made, no histopathological finding could be noted indicating carcinogenic action on the renal tissue by DES administered to postnatal rats. However, structural alterations, particularly in the glomerular capillaries and proximal tubuli, parallel to the increase in dosage in all the three experimental groups were observed.

The dilatation of the glomerular capillaries, closing of the urinary space and increase in the filtering membrane disorders due to increase in dosage seem to emphasize Na/H_2O balance disorder in the tissue and change in the ultrafiltration.

Ultrastructural findings stressing this functional pathology were indicated by failure to trace the fenestrae belonging to the endothelial cells of the glomerular capillaries, loss of homogenous appearance of the basement membrane displaying ondulation and thickening in many areas and deterioration of the slit membrane due

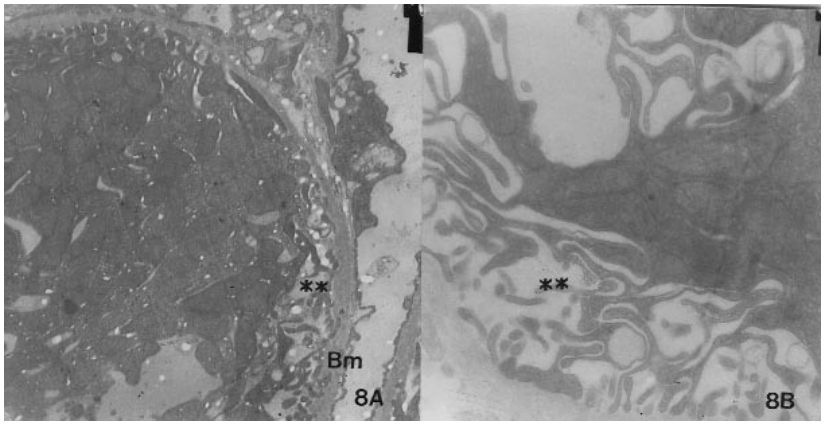

Figure 8A-8B. The proximal tubulus belonging to Group III. In this electron micrograph, increased basement membrane (Bm) thickening and dilatation in the intracytoplasmic foldings (**) could be seen. 8A: X10.000 8B: X18.000

to the exfoliation of the podocytes (Figs. 3,5,7).

In investigations made, it has been observed the proximal tubulus cells directed to estrogens in hamsters administered DES or cultured renal tissues displayed a proliferation. Depending on the dosage period, it has been demonstrated that this effect increased and cancer cells developed in a solitary manner or in bundles (9,10). There was a definite elevation in the plasma renin activation (PRA) measured in the same investigation (9,10).

In this research work undertaken, it has been observed in the investigations made on the tubular area of the cortex that the distal tubulus cells preserved their morphological structures. But, histopathological findings obtained parallel to an increase of dosage in the proximal tubulus were noted. Cells with picnotic nuclei were encountered from place to place in the proximal tubulus and thickening of the basement membrane and a marked dilatation in the intracytoplasmic foldings were observed (Figs. 4A,4B,6A,6B,8A). The mitochondria taking place between these foldings had electron-dense structures (Fig. 8A). When considered that this structural features localized in the basal region of the proximal tubulus cells plays a role in the Na pump, the histopathological alterations taking place together with those determined in the glomeruli appear to be significant.

In conclusion, although these effects caused by DES are far from defining carcinoma, a functional disorder is clearly suggested. There is, however, a need for further investigations to establish whether these functional disorders determined in this study suggest that DES plays a procarcinogenous role on tissues, including the rat kidney in face of previous evidence that no cancer formation has thus far been noted morphologically.

References

1. Marselos M, Tomatis L. Diethylstilbestrol I: Pharmacology, toxicology and carcinogenicity in humans. Eur J Cancer 28: 1182-89, 1992.
2. Marselos M, Tomatis L. Diethylstilbestrol II: Pharmacology, toxicology and carcinogenicity in experimental animals. Eur J Cancer 29 : 149-55, 1992.
3. Kohygashy K, Fukuda Y, Imura H. Inhibitory effect of tamoxifen on diethylstilbestrol-promoted hepatic tumorigenesis in male rats and its possible mechanism of action. Jpn J Cancer Res 79: 1135-39, 1988.
4. Şener S. Residues in animal products. Veterinary and Animal Husbandry Research Group Specialty Committee Report, The Scientific and Technical Research Council of Turkey, 114-22, 1994.
5. Zimmerman AS, Clevenger WR, Brimhall BB, Bradshaw SW. Diethylstilbestrol-induced perinatal lethality in the rat. II. Perturbation of perturition. Biol Reprod 44: 583-9, 1991.
6. Clevenger RW, Cornwal AG, Carter MW, Broadshaw SW. Diethylstilbestrol-induced perinatal lethality in the rat. I. Relationship to reduced maternal weight gain. Biol Reprod 44: 575-82, 1991.
7. Newbold R, Jellinck PH, Metzler M, McLanchlan JA. Ontogeny of peroxidase activity in epithelium and eosinophils of mouse uterus. Teratogenesis carcinog mutagen 11: 267-78, 1992.
8. Hey M, Haaf H, McLanchlan JA, Metzler M. Indirect evidence for the metabolic dehalogenation of tetrafluorodiethylstilbestrol by rat and hamster liver and kidney microsomes. Species and organ dependent differences. Biochem Pharmacol 35: 2135-39, 1986.

9. Oberley TD, Lauchner JL, Pugh DT, Gonzales A, Goldfarb S, Ly SA, Ly JJ. Specific estrogen-induced cell proliferation of cultured Syrian hamster renal proximal tubular cells in serum-free chemically defined media. Proc Natl Acad Sci 86: 2107-11, 1989.

10. Dodge A, Brownfield M, Reid Al, Inagami T. Immunohistochemical renin study of DES, induced renal tumor in the Syrian hamsters. Am J Anat 182: 347-52, 1988.

11. Fores F, Bar-Ami S, Haj-Yehia Y, Gravish M. Hormonal regulation of peripheral benzodiazepine binding sites in female rat adrenal gland and kidney. J Recept Res 9: 143-57, 1989.

12. Williams GM, Latropoulos M, Cheung R, Radi L, Wang CX. Diethylstilbestrol liver carcinogenicity and modification of DNA in rats. Cancer Letter 68: 193-8, 1993.

13. Braugler JM, Gilloteaux J, Steggles AW. Alterations in the subcellular distribution of Guanylate cycylase and its responsiveness to nitric oxide in DES-induced renal tumors. Cancer 50 : 78-84, 1982.

14. Reynolds EA. The use of lead citrate at high pH as an electron-opaqe stain in electronmicroscopy. J Cell Biol 17: 208-12, 1963.

15. Katayama S, Lee JB. Estrodiol stimulates rat renopapillary prostaglandin E2, but not PGF2a biosynthesis. Endocrinol 117: 656-61, 1985.

16. Kinne E, Kinne P. Proton pump activity and Mg-ATPase activity in rat kidney cortex brushborder membranes. Pflugers Arch 407, Suppl 2F, 180-5, 1986.

17. Walsh JC, Berry SJ. Estrogen enhancement of androgen-induced renal growth in the male rat. J Urol 137: 159-62, 1987.

18. Haaf H, Metzler M. In vitro metabolism of DES by hepatic, renal and uterine microsomes of rats and hamster. Biochem Pharmacol 34: 3107-15, 1985.