EFFECTS OF PROLONGED ADMINISTRATION OF DELTA -9- TETRAHYDROCANNABINOL IN RAT KIDNEY CELLS

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SUMMARY: Kidneys of tetrahydrocannabinol (THC) treated rats were studied by electron microscope and compared to those of normal controls. The experimental group received THC for five weeks twice daily while control animals received saline. The most important differences between these two groups were observed in lysosomes. In addition to these minor changes several large secondary lysomes were observed in the cells of proximal convoluted tubules of the delta -9- THC treated rats. The mitochondria of the experimental group were also degenerated in some cells. The morphology of filtration barrier however appeared normal in glomeruli of THC treated rats.

Key Words: Delta -9 Tetrahydrocannabinol, Glomerulus, Proximal Convoluted Tubules, Mitochondria, Lysosomes.

INTRODUCTION

Hashish is an extract containing only the drug-rich resin secreted by the hemp plant. Chemists have been able to isolate more than twenty compounds from specimens of Cannabis sativa (9). These compounds are collectively referred to as the cannabinoids. Numerous studies have indicated that delta-9-tetrahydro-cannabinol (THC) is the major psychoactive compound in marihuana and hashish. The effects of delta -9- tetrahydrocannabinol were examined previously in different organs especially in liver and brain (3-5, 10,13, 22, 23).

Regardless of whether THC is administrated by the oral route, by the intravenous route, or by inhalation, the greater part of its metabolites is found in the feces and to a lesser extent in the urine (18,21). A variety of analytic techniques have been used to measure delta -9- THC and its metabolites in the urine (2,9). A review of the medical literature revealed that there are no publications about delta-9-THC induced electron microscopic changes in rat kidney cells. The present study was especially designed to characterize the effects of prolonged administration of delta -9-THC on the mitochondria and lysosomes of the rat kidney cells.

MATERIALS AND METHODS

Twenty female rats, Weighing 100-120 gr, were used in this study. Since the details of treatment and electron microscopic methods have been published previously (12,13), only some important features of the procedure will here be mentioned. Animals were randomly divided into two groups. Ten rats were injected s.c. with THC as follows: first week -10 mg/kg twice daily; second week -20 mg/kg twice daily. And thereafter, for three weeks they received 40 mg/kg THC twice daily. Ten animals served as controls. They received similar concentrations and volume of Tween-20 and alcohol in saline twice daily, for the same period of time (12,13). The animals were sacrificed 3 days after the last injection.

Kidneys were cut into small pieces and were fixed in a 2.5% 4°C temperature. The specimens were then postfixed with 1% osmium tetroxide solution at pH 7.4 for one hour. They were later dehydrated by graded concentrations of ethanol. During dehydratation, samples were keeping them for one hour in a saturated uranyl acetate solution in 70% ethanol. After dehydration, the tissues were embedded in araldite (CY 212). Tnin sections were made with glass knives by LKB ultramicrotome and were examined by Carl Zeiss E. M. 9S.

RESULTS

The ultrastructure of the glomerulus of the proximal convoluted tubules (PCT) were found normal in rat kidney sections of the solvent treated control groups (Figure 1,3). In the glomerulus, the capilaries are lined by a thin layer of fenestrated endothelial cytoplasm. A thick basement membrane interposed between the fenestrated capillary endothelium on the inside and foot processes of the podocytes on the outside (Figure 1). Several capillary loops recognized by their content of erytrocytes and precipitated plasma proteins (Figure 1).

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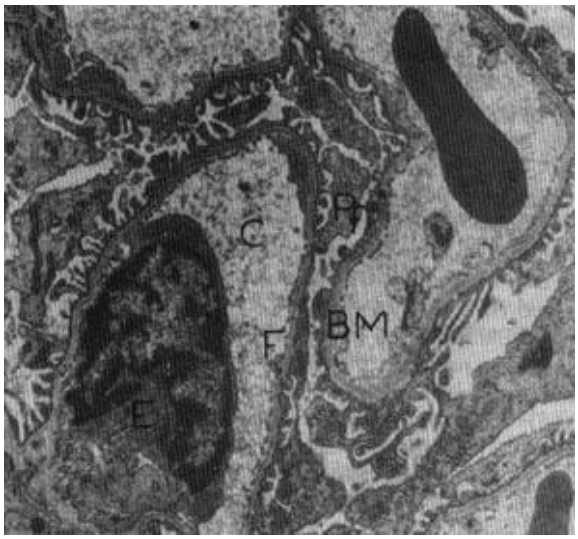

Figure 1: The normal structure of the glomerulus of the control group of rats are seen where the capillaries are lined by a thin layer of fenestrated endothelial cytoplasm. An endothelial cell nucleus is seen builging into the capillary lumen (E). Fenestrated endothelium is covered by a thick basal membrane. Foot processes of the podocytes rest on the outside of glomerular basement membrane. X12 000.
E: Endothelial cell, C: Capillaries, F: Fenestrations, Pf: Foot processes of podocytes, BM: Basement membrane

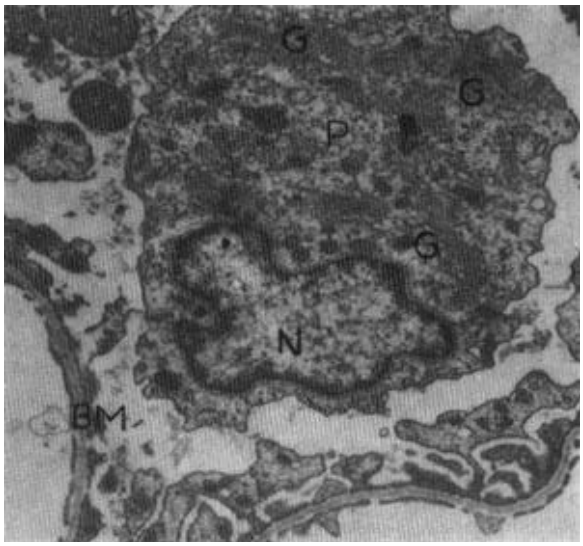

Figure 2: Glomerulus of the delta -9- THC treated rat kidney. A podocyte (P) and two capillary lumens are seen in this micrograph. Podocyte has an eccentric nucleus with several identations. It has several Golgi complexes. The architecture of the glomerular filtration membrane is normal. X 18 800.
P: Podocyte, G: Golgi complex, N: Nucleus, BM: Basal membrane

The ultrastructure of the glomerulus of the delta-9-THC treated rat kidney is seen with normal structure. Electron micrograph of the filtration barier is also seen in normal structure (Figure 2). A podocyte has an eccentric nucleus with several indentations. It has several Golgi complexes, mitochondria and free ribosomes in their cytoplasm (Figure 2).

Electron micrograph of a proximal convoluted tubule (PCT) of control rats has the cells with normal structure (Figure 3). The basal plasma membrane of the PCT exhibits deep basal infoldings into the cell. Several mitochondria are concentrated at the base of the cell and are arranged to the long axis of the cell (Figure 3).

In the electron micrographs of the PCT of the delta -9-.THC treated rat kidney, there are many cells having many vacuoles and several large dense granules in their cytoplasm (Figure 4). Dense granules are thought to be secondary lysosomes (Figure 5). The morphology of the mitochondria is seen in the normal contour (Figure 5). But there are swellings and degenerations of mitochondria of some cells seen near distal convoluted tubule (DCT) cells.

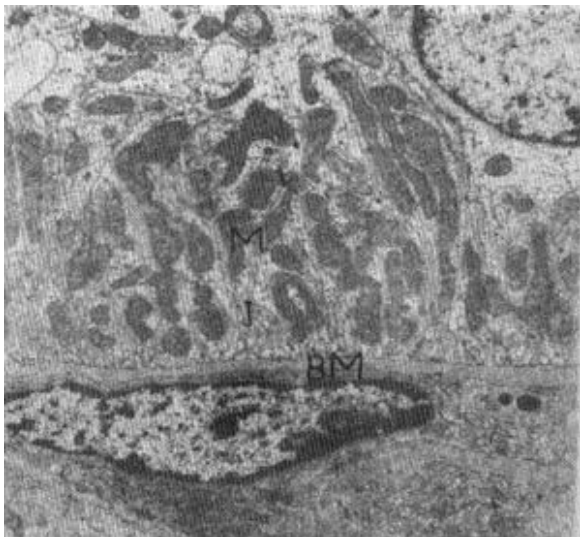

Figure 3: Electron micrograph of the basal part of a proximal convoluted tubule (PCT) of a control group rat kidney where deep basal infoldings into the cell are observed. Mitochondria are concentrated at the base of the cell and arrenged parallel to the long axis. Note the basement membrane separating the base of the tubule lining cells from the outside. X34 000.
M: Mitochondria, I: Infoldings, BM: Basement membrane

DISCUSSION

One finds that, when the THC contained a radioactive label, 15 to 30% in the urine of cannabinoids is excreted in the course of a-day period (21). Very little unchanged

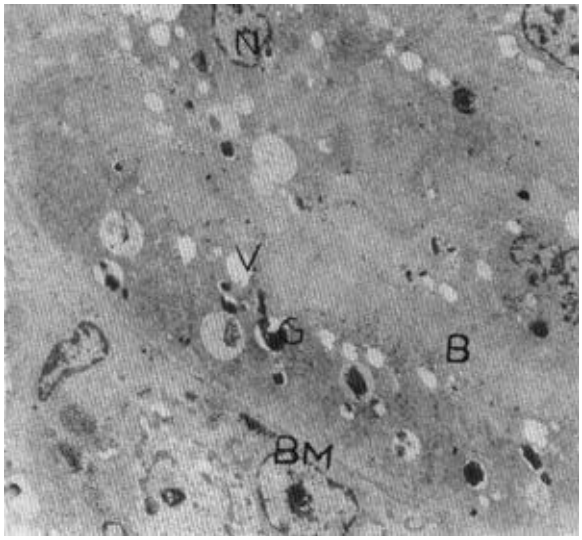

Figure 4: Electron micrograph of a proximal convoluted tubule (PCT) of the delta -9-THC treated rat kidney. Many vacuoles and several large dense granules are seen in the cytoplasms of the PCT cells. It is believed that dense granules represent secondary lysosomes. X 7600
N: Nucleus, V: Vacuoles, G: Granules, B: Brush border, BM: Basement membrane.

THC is found in either urine or the feces. When THC bearing a radioactive label of ^{3}H or ^{14}C is administrated to human subjects, about three-quarters of the urinary radioactivity is associated with compounds of unknown structure (21).

These findings suggest that, if human beings or an animal are given THC, some morphological changes in kidney would be expected. The effects of hashish compounds on rat liver, especially on rat liver mitochondria has been previously described (3,4,10,13,19). It has been shown that 1.5 hour after intraperitoneal injection of ^{14}C-tetrahydrocannabinol, most of the radioactivity accumulates in the liver (18). The main alterations are swellings in mitochondria of the liver cells and inner mitochondrial structures are disrupted (3,13,19). In some liver cells, the lysosomes and lipofuscin pigments were observed (13).

The ultrastructure of glomerular barrier and podocytes were normal in the delta-9-THC treated rats. Mitochondria appeared normal in structure in the PCT cells of the THC treated rat kidneys. But, swellings and disruption of inner mitochondrial structure were observed in the cytoplasm of some cells near DCT of the THC treated rats, giving the impression of the impending degeration of the entire cell.

Most important differences were observed on the lysosomes of the proximal convoluted tubule (PCT) cells of the

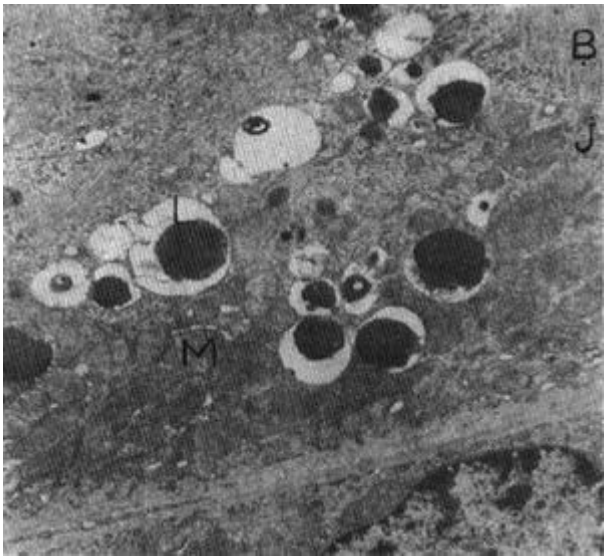

Figure 5: High magnification of a proximal convoluted tubule cell of the delta -9-THC treated rat kidney. Many large secondary lysosomes are seen in the cytoplasm. Note the normal contour and morphology of the mitochondria. X 34000
B: Brush border, J: Junctional complex, L: Lysosomes, M: Mitochondria.

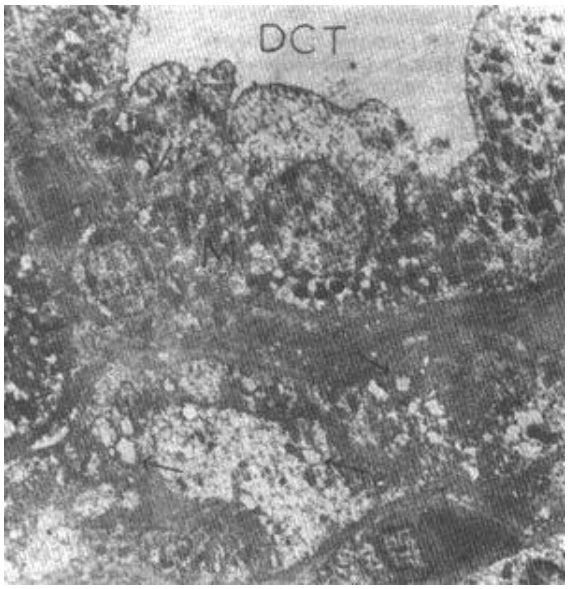

Figure 6: Electron micrograph of a distal convaoluted tubule (DTC) of the delta -9-THC treated rat kidney. Many vacuoles and mitochondria are generally seen together in the cytoplasm of the DCT cells. Several swollen and degenerated mitochondria are observed in the cytoplasm of neighboring cells. X7600
DCT. Distal convoluted tubule, V: Vacuoles, M: Mitochondria, Arrows: Swollen and degenerated mitochondria.

delta-9-THC treated rats. Many large lysosomes were found on the PCT cells of the THC treated rats.

Irvin and Mellors (10) have reported that liver lysosomes become highly labeled with ^{14}C-radioactivity compared to other subcellular fractions in the ^{14}C - delta - 9 - THC radioactivity is taken up rapidly by the liver, so that the whole liver contains at 5 and 15 samples 8.7 and 15.5% of the total dose give respectively (10). All other subcellur fractions show lower uptakes of radioactivity. Lysosomes, however, have a specific activity five times that of the homogenate within 5 min of the injection of ^{14}C-delta-9-THC. This specific activity for the isotope in lysosomes increases to a maximum of 17 fold compared to the homogenate at 18 hours after the injection, and thereafter the specific activity declines slowly (4,10,22).

In the present study, apical vesicles and large lysosomes increased on the PCT cells. Previous studies have indicated that delta-9-THC and related cannabinoids have disruptive effects on rat liver lysosomes (4,22). It has been estimated that lysosomes can lyse and degerate cells of PCT of animals given delta-9-THC chronically.

REFERENCES

1. Abel EL, SE Tan, M Subramanian: Effects of delta-9-tetrahydrocannabinol, phenobartial and their combination of pregnancy and offspring in rats. Teratology 36:193-198, 1987.

2. Alburges ME, MA Peat : Profiles of delta-9-tetrahydrocannabinol metabolites in urine of marijuana users. Journal of Forensic Sci 31:695-706, 1986.

3. Bino T, A Chari-Bitron, A Shahar: Biochemical effects and morphological changes in rat liver mitochondria exposed to delta -1-tetrahydrocannabinol. Biochim Biophys Acta 288:195-202, 1972.

4. Britton RS, A Mellors : Lysis of rat liver lysosomes in vitro by delta-9-tetrahydrocannabinol. Biomedical Pharmacology 23:1342-1344, 1974.

5. Chakravarty I, VI Naik : Action of delta-9-tetrahydrocannabinol on the binding of estradiol to uterine and other tissues in rats. Biochemical Pharmacology 32:253-256, 1983.

6. Cutter MG, JH Mackintosh . Cannabis and delta-9-tetrahydrocannabinol. Effects on elements of social behaviour in mice. Neuropharmacology 23:1091-1097, 1984.

7. Erbengi T, M Clara : Systema Urýgenitale. In Histoloji atlasi Istanbul Un. Istanbul Tip Fakültesi, 2720/124, p 186-203, 1980.

8. Fowcett DW : The Urinary System. In A Textbook of Histology. Philadelphia, 11th Ed WB Saunders Co, p 755-780, 1986.

9. Hofmann FG: The Cannabinoids: Absorbtion, Biotransformation, and Excretion. In A Handbook on Drug and Alcohol Abuse, The Biomedical Aspects. 2nd Ed, New York, Oxford University Press, p 123-203, 1983.

10. Irvin JE, A Mellors: Delta-9-tetrahydrocannabinol -up-take by rat liver lysosomes. Biomedical Pharmacology 24:305-307, 1975.

11. Junqueira LC, I Carneiro, JA Long: Urinary System. In Basic Histology. Los Altos, California, Lange Medical Publications, p 413-433, 1986.

12. Kaymakçalan S, IH Ayhan, FC Tulunay: Naloxone -induced or postwithdrawal abstinence signs in delta -9-tetrahydrocannabinol -tolerant rats. Psycopharmacology 55:243-249, 1977.

13. Kaymakçalan S, I Kerse, Ü Örs, A Özyazici, D Balta: Delta-9-tetrahydrocannabinol induced electron microscopic changes in rat liver cells. Hacettepe Bull Med Surg 11:38-44, 1978.

14. Lindgren JE, A Ohlsson, S Agurell: Clinical effects and plasma levels of delta-9-tetrahydrocannabinol in heavy and light users of cannabis. Psychopharmacology 74:208-212, 1981.

15. McMillan DE, WC Hardwick, JD Wells: Effects of Barbiturats in Rats Tolerant to delta-9-tetrahydrocannabinol. Arch Int Pharmacodyn 264:4-14, 1983.

16. Mechoulam R, A Shani: Chemical Basis of Hashish Activity. Science 169:611-612, 1970.

17. Miczek KA, BN Dixit : Behavioral and Biochemical Effects of Chronic Delta -9-tetrahydrocannabinol in Rats. Psychopharmacology 67:195-202, 1980.

18. Miras CJ : Hashish, its Chemistry and Pharmacology. Ciba Foundation Study, Group No: 21, p 37-41, 1965.

19. Mahoney JM, RA Harris : Effect of Delta -9-tetrahydrocannabinol on Mitochondrial Procecesses. Biochemical Pharmacology 21:1217-1226, 1972.

20. Nicolau M, AJ Lapa, JR Wolle : The inhibitory effects induced by delta-9-tetrahydrocannabinol on the contractions of the isolated vas deferens. Arch Int Pharmacodyn 236:131-136, 1978.

21. Perez-Reyes M, MA Lipton: Pharmacology of orally administrated delta-9-tetrahydrocannabinol. Clin Pharmacol Therap 14:48-53, 1973.

22. Raz A, A Schurr: Effect of hashish compounds on rat liver lysosomes in vitro. Biochemical Pharmacol 22:3129-3131, 1973.

23. Rosenkrantz H, DW Hayden: Acute and Subacute Inhalation Toxicity of Turkish Marihuana, Cannabichromene, and Cannabidiol in Rats. Toxicol Appl Pharmacol 48:375-386, 1979.

24. Semjonow A, M Binder: Generalization of the discrimintive stimulus properties of delta-9-THC to delta-9 (11)-THC in rats. Psycopharmacology 85:178-183, 1985.

25. Stiglick A, H Kalant : Behavioral effects of prolonged administration of delta-9-THC in the rat. Psychopharmacology 80:325-330, 1983.

26. Ülkü E, IH Ayhan, FC Tulunay, B Uran, S Kaymakçalan : Effect of delta -9-THC on the morphine-induced hyperactivity of mice. Psycopharmacology 69:201-205, 1980.

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