Research Articles

Effect of the synthetic cannabinoid nabilone on spermatogenesis in mice

P. B. Patra [1] and R. M. Wadsworth [2]

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW (Scotland)
Received 31 August 1989; accepted 9 May 1990

Summary. Chronic treatment of mice with the synthetic cannabinoid nabilone (50 mg/kg, 3 times per week) reduced the number of pachytene spermatocytes. Nabilone did not affect other cell types in the testis or the sex organ weights. Nabilone tended to increase the number of abnormal spermatozoa, but this did not reach statistical significance. It is concluded that nabilone causes less testicular toxicity than the natural cannabinoids.
Key words. Nabilone; spermatogenesis; cannabinoid.

In recent years there has been a growing realization of the therapeutic potential of cannabinoids [3] especially in the control of nausea [4] and glaucoma [5]. However, delta-9-tetrahydrocannabinol (THC) and other naturally occurring cannabinoids are not suitable for therapeutic use because of their central nervous system effects and abuse potential. Several synthetic cannabinoids have been developed with more specific actions [6, 7], the most promising of which is nabilone [8], an effective antiemetic drug for moderate toxic chemotherapy [9]. It is well established that prolonged use of marihuana leads to a variety of derangements in the reproductive system, including disturbance of spermatogenesis, abnormalities in sperm morphology and alterations of hormonal levels in animals and man [10]. As the effects of nabilone on the male reproductive system have not been investigated in detail, we have in the present study examined its effect on spermatogenesis on mice. A preliminary version of this data has been presented [11].

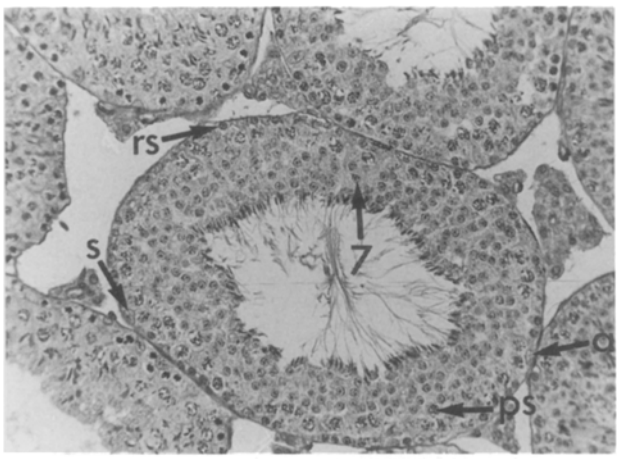
Materials and methods

Male, 6-week-old BKA mice were maintained on a 12 hour light/dark cycle and supplied with food and water ad libitum. The mice were divided into five groups: a) untreated, b) vehicle treated controls for 35 days, c) nabilone (50 mg/kg) for 35 days d) vehicle treated controls for 70 days e) nabilone (50 mg/kg) for 70 days. Nabilone was suspended in a mixture of alcohol, polysorbate 20 and water (10 : 1 : 89) and administered orally 3 times a week (volume of vehicle or nabilone suspension administered = 5 ml/kg). Following oral administration, nabilone is rapidly absorbed and bound in the tissue, being subsequently eliminated as metabolites with a half-life of 30 h [12]. Animals were killed at 35 days or 70 days after commencement of treatment (mouse spermatogenic cycle = 34–35 days), the sex organs and adrenals were weighed and the testes were fixed in Bouin's fixative. Sections were cut at 5 μm and stained with periodic acid-Schiff-haematoxylin. Spermatogenesis was quantified by counting the number of germ cell nuclei in each of 20 circular stage VII tubules as described by Oakberg [13] (n = 6 mice). Counts were corrected for differences of nuclear diameter according to Abercrombie [14] and for tubular shrinkage using the Sertoli cell correction factor of Clermont and Morgentaler [15]. To detect the effects of nabilone on mitosis, the total number of type A spermatogonia were also counted at stages I, II, IV and XI when the peaks of mitosis occur in the mouse [16]. Approximately 25 round Leydig cell nuclei adjacent to the stage VII tubule were drawn using a camera lucida and their area measured (n = 6 mice). Smears of cauda epididymal spermatozoa were prepared as described by Taylor [17]. 200 spermatozoa from each animal were examined and abnormal morphology classified according to the criteria of Wyrobek and Bruce [18]. Statistical analysis was done using Student's unpaired t-test.

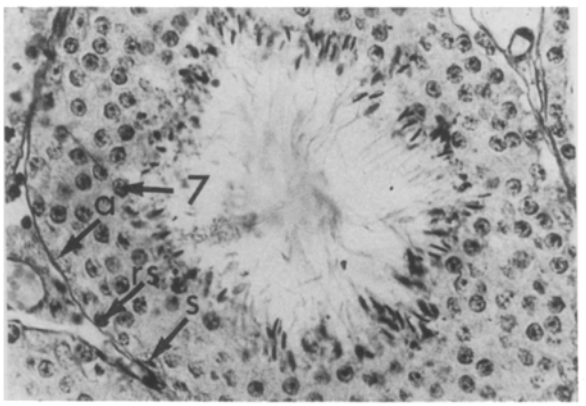
Results and discussion

Nabilone had no effect on body weight, nor on the weights of the paired testes, epididymides, seminal vesicles or adrenals at either 35 or 70 days. The weight of the paired ventral prostates was significantly reduced after 35 days treatment (vehicle = 48 ± 3 mg, nabilone = 38 ± 2 mg), although this effect could not be demonstrated when ventral prostate weight was normalized for body weight (vehicle = 110 ± 5, nabilone = 98 ± 7 mg/100 g b.wt). Ventral prostate weight was not affected in the group treated with nabilone for 70 days.

Nabilone produced severe degeneration in the testis especially after 70 days' treatment. In some tubules there was complete absence of pachytene spermatocytes observed at stage VII of spermatogenesis (fig. 1 b) and in others there was pyknosis. These degenerative changes were reflected in a reduction in the pachytene spermatocyte numbers which occurred after 70 days treatment. The vehicle itself also reduced the resting and pachytene spermatocyte numbers in comparison to untreated control animals. Nevertheless the effect of nabilone is significantly greater than that of the solvent (fig. 2). Nabilone had no effect on the number of type A spermatogonia, or resting spermatocytes or step 7 spermatids (fig. 2).



A



B

Figure 1. Photomicrograph of mouse testis at stage VII of a seminiferous tubule. a = type A spermatogonia, rs = resting spermatocyte, ps = pachytene spermatocyte, 7 = step 7 spermatids and s = Sertoli cell. *A* Untreated control showing the normal cellular association at stage VII. *B* Mouse treated for 70 days with nabilone showing complete absence of pachytene spermatocytes in a stage VII seminiferous tubule.

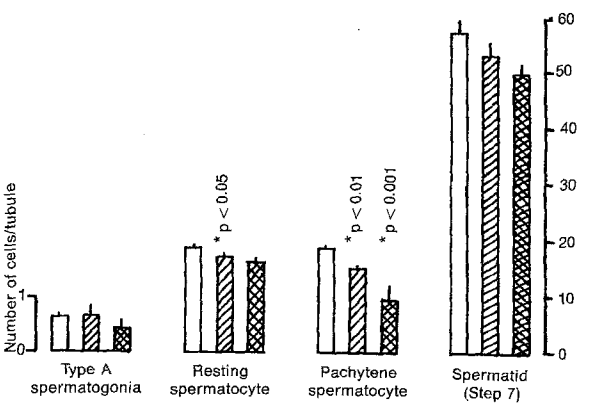


Figure 2. Quantitification of spermatogenesis of mice after nabilone treatment for 70 days. Crude cell counts at stage VII of spermatogenesis were corrected for nuclear diameter and by the Sertoli cell number. The left scale indicates type A spermatogonia and the right scale indicates the number of resting spermatocytes, pachytene spermatocytes and step 7 spermatids. Vertical bars on each column represent the SE mean, $n = 6$. The level of significance was calculated using Student's unpaired t-test comparing vehicle control with untreated control group and nabilone with the vehicle control group. □ Untreated; ▨ Vehicle; ▩ Nabilone.

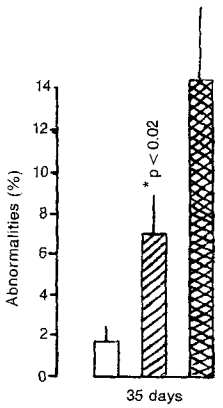


Figure 3. Effect of nabilone on sperm morphology after 35 days' treatment in mice. The vertical bars on each column represent the SE mean, $n = 6$. The level of significance was calculated using Student's unpaired t-test comparing vehicle control with untreated control group and nabilone with the vehicle control group. □ Untreated; ▨ Vehicle; ▩ Nabilone.

Nabilone treatment for 35 days had no significant effect on the number of type A spermatogonia, resting and pachytene spermatocytes or step 7 spermatids. Nabilone had no effect on the frequency of any of spermatogenic stages I–XII or on Leydig cell nuclear area either after 35 or 70 days treatment. The vehicle for nabilone produced a significant increase in the number of abnormal spermatozoa in comparison to untreated control animals (fig. 3). There was a tendency for nabilone treatment to further increase the number of abnormal spermatozoa but this did not reach significance in comparison to vehicle.

In this study, twice weekly chronic administration of nabilone to mice reduced the number of pachytene spermatocytes. Pachytene spermatocytes are very sensitive to inhibitors of testicular function; for example, the alkylating agent triethylenemelamine acts on the more mature germ cells in rats[19]. The effect of nabilone is similar to that seen with cannabinol, cannabidiol and tetrahydrocannabinol[20] suggesting that all cannabinoids can inhibit one particular stage of germ cell division. This effect could explain the antifertility action reported with nabilone in male rats[21]. Nevertheless, the effect of nabilone is much more limited than the effects of the natural cannabinoids, which reduce type A spermatogonia, resting and pachytene spermatocyte numbers, increase Leydig cell nuclear area and increase in the percentage of sperm with abnormal morphology. However, the possibility should still be borne in mind that nabilone may disturb testicular function after prolonged use. The tubular shrinkage and abnormalities in sperm morphology produced by the vehicle is probably attributable to the ethanol (the dose of which was 1 ml/kg, 3 times a week) as has been reported in rats[22–24].

1 Present address: Dept of OB-GYN, Duke University Medical Center, Durham, NC, USA. PBP was supported by an ORS award and David Livingstone Bursary from the U.K.

2 To whom all correspondence should be addressed.
3 Bhargava, H. N., Gen. Pharmac. *9* (1978) 195.
4 Gralla, R. J., Tyson, L. B., Bordin, L. A., Clark, R. A., Kelson, D. P., Kalman, L. B., and Groshen, S., Cancer Treat Rep. *68* (1984) 163.
5 Green, K., in: Applied Pharmacology on the Medical Treatment of Glaucoma, p. 507. Ed. S. M. Drance. Grune and Stratton, Inc., 1984.
6 Razdan, R. K., and Howes, J. F., Med. Res. Rev. *3* (1983) 119.
7 Mechoulam, R., in: Cannabis as Therapeutic Agents. CRC Press, Inc., 1986.
8 Lemberger, L., A. Rev.. Pharmac. Toxic. *20* (1980) 151.
9 Vincent, B. J., McQuiston, D. J., Einhoorm, L. H., Nagy, C. M., and Brames, M. J., Drugs *25*, suppl. 1 (1983) 52.
10 Abel, E., Drug Alcohol Depend. *8* (1981) 1.
11 Patra, P. B., and Wadsworth, R. M., in: Marihuana '84, Proceedings of the Oxford Symposium on Cannabis, p. 287. Ed. D. J. Harvey. IRL press, Oxford 1984.
12 Lemberger, L., Rubin, A., Wolen, R., DeSante, K., Rowe, H., Forney, R., and Pence, P., Cancer Treatment Rev. *9*, suppl. B (1982) 17.
13 Oakberg, E. F., Am. J. Anat. *99* (1956) 391.
14 Abercrombie, M., Anat. Rec. *94* (1946) 239.
15 Clermont, Y., and Morgentaler, H., Endocrinology *57* (1955) 369.
16 Oakberg, E. F., Am. J. Anat. *99* (1956) 507.
17 Taylor, N. J., Stain Technol. *57* (1982) 245.
18 Wyrobek, A. J., and Bruce, W. R., Proc. natl Acad. Sci. USA *72* (1979) 4425.
19 Steinberger, E., J. Reprod. Fert. *3* (1962) 250.
20 Patra, P. B., and Wadsworth, R. M., Society for the Study of Fertility Meeting, London, December 1985.
21 Markham, J. K., Hanasono, G. K., Adams, E. R., and Owen, N. V., Toxic. appl. Pharmac. *48* A119 (1988) 238.
22 Klassen, R. W., and Persaud, T. V. N., Int. J. Fert. *23* (1978) 176.
23 Anderson, R. A. Jr, Willis, B. R., Oswald, C., and Zeneveld, L. J. D., J. Pharmac. exp. Ther. *225* (1983) 479.
24 Weinberg, J., and Vogl, A. W., J. Androl. *9* (1988) 261.

0014-4754/90/080852-03$1.50 + 0.20/0

Superoxide dismutase activity in the Spanish population

M. R. De la Torre, A. Casado and M. E. López-Fernández

U.E.I. de Citogenética del Centro de Investigaciones Biológicas del Consejo Superior de Investigaciones Científicas, c/Velázquez 144, E-28006 Madrid (Spain)
Received 18 September 1989; accepted 24 January 1990

Summary. Superoxide dismutase is an enzyme that catalyzes the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. This superoxide radical is produced by all aerobic cells as a normal metabolic intermediate of molecular oxygen, and is dangerous for the cell because it induces the inactivation of various enzymes, lipid peroxidation and mutations. Superoxide dismutase can therefore be considered as a protective enzyme. The purpose of this work was to determine the level of superoxide dismutase activity in the Spanish population, and to study the factors that influence this activity. The superoxide dismutase activity of 2397 individuals was determined using the method described by Minami and Yoshikawa. The superoxide dismutase activity level in the adult Spanish population was found to be 4.16 ± 0.89 Units/ml of blood. No significant variations with respect to sex were detected. But it was observed that the superoxide dismutase activity level was 9% higher in the young urban Spanish population.
Key words. Superoxide; superoxide dismutase; sex; age; aging processes.

There is an enzymatic activity universally present in all aerobic cells. The substrate is an unstable free radical that can be present only in minuscule amounts at any instant, and the reaction catalyzed proceeds at a rapid rate even in the absence of the enzyme. Yet the enzyme, superoxide dismutase, is essential for the survival of aerobic cells. It catalytically scavenges the superoxide radical, which is an important agent of the toxicity of oxygen, and thus provides a defense against this aspect of oxygen toxicity[1].

Superoxide radical can act either as a reducing agent, giving up its extra electron, or as an oxidizing agent, becoming reduced to hydrogen peroxide. For example, it reduces cytochrome c, but it oxidizes molecules such as ascorbic acid and adrenalin. It can also decarboxylate ketoacids and react with certain phenols. In comparison with other oxygen radicals, superoxide is rather unreactive, with a lifetime of milliseconds at physiological pH values. But superoxide can react with hydrogen peroxide to produce hydroxyl radicals and excited state oxygen (singlet oxygen $^1\Delta$ g), which are among the most reactive species known to organic chemistry; they will attack and damage almost every molecule found in living cells. For example, they can attack olefinic bonds and so initiate lipid peroxidation. Once initiated, lipid peroxidation is autocatalytic. Lipid peroxides are powerful inhibitors of many enzymes, causing severe damage to membranes and eventual loss of membrane integrity. Hydroxyl radicals and singlet oxygen can also hydroxylate the purine and pyrimidine bases present in DNA, resulting in mutations[2].

Superoxide radical is a common intermediate of oxygen reduction. A number of reactions of biochemical interest have been shown to generate superoxide radical. Among these are the autoxidation of hydroquinones, leucoflavins, catechol amines, reduced ferredoxins, hemo-