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MARIJUANA EFFECTS ON IMMUNITY: SUPPRESSION OF HUMAN NATURAL KILLER CELL ACTIVITY BY DELTA-9-TETRAHYDROCANNABINOL

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Abstract—Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, was tested for its ability to modulate human natural killer (NK) cell function. THC was toxic for peripheral blood lymphocytes at 20 μg/ml but not at 10 μg/ml or less. This component of marijuana also was inhibitory for NK activity against K562, a human tumor cell line at concentrations down to 5 μg/ml when pre-incubated with the effector cells. Suppression of NK function was dependent upon the concentration of THC and the length of time of pre-incubation but was independent of the ratio of effector to target cells. Prostaglandins were not involved in suppression of NK activity.

The psychoactive effects of marijuana, as well as some of the physiological and biochemical effects of this drug, are well documented. There is considerably less information, however, concerning the ability of marijuana and its components, such as delta-9-tetrahydrocannabinol (THC), to influence various aspects of the immune system. Acute or chronic exposure of human or mouse peripheral blood leukocytes (PBL) to marijuana have yielded equivocal results when these cells were exposed to specific (antigens) or nonspecific (plant mitogens) stimuli (Matsuyama & Jarvik, 1977; Munson & Fehr, 1983; White, Brin & Janicki, 1975). A number of reports have appeared indicating that marijuana has detrimental effects on immunity, suppressing anitbody responses (Levy, Farrar, Harris, Dewey & Munson, 1975; Levy & Heppner, 1981; Rosenkrantz, Miller & Esber, 1975), cell-mediated immune responses and macrophage function (Huber, Pochay, Pereira, Shea, Hinds, First & Sornberger, 1980; Levy & Heppner, 1979; Nahas, Suciu-Foca, Armand & Morishima, 1974), but there are a number of other reports indicating no effects of THC on these responses (Lau, Tubergen, Barr, Domino, Benowitz & Jones, 1976; Peterson, Graham & Lemberger, 1976; Rachelefsky, Opelz, Mickey, Lessin, Kiuchi, Silverstein & Stiehm, 1976).

Natural killer (NK) cells, which are recognized as being important in host defenses against tumor cells and microbial infections, have not been examined for their susceptibility to modulation by THC. These cells are considered a first line of defense against infections and often inhibit tumors before they can become well established. NK cell function has been demonstrated to be influenced by a variety of substances which inhibit or enhance imunologic responsiveness (Brunda, Herberman & Holden, 1980; Droller, Schneider & Perlman, 1978; Henney, Kuribayashi, Kern & Gillis, 1981; Oehler, Lindsay, Nunn, Holden & Herberman, 1978). Prostaglandins and corticosteroids clearly inhibit NK function, while interferons and interleukins enhance activity. Considering the importance of NK cells in host resistance, it was of interest to examine the effects, if any, of THC on NK activity. The results of the present study indicate that THC suppresses human NK cell activity *in vitro*.

EXPERIMENTAL PROCEDURES

Blood donors

Healthy donors who denied a history of marijuana use served as a source of PBL for these studies. Thirty ml blood were collected by venepuncture and

Table 1. Cytotoxicity of THC over a 3 h time period for human peripheral blood leukocytes

Addition to cultures*	Mean percent cytotoxicity after treatment for† 30 min	60 min	120 min	180 min
Medium	4.0*	6.7	10.5	13.9
DMSO (0.1%‡)	4.2	8.4	9.7	16.5
THC (μg/ml)				
20	21.9	36.6	40.5	51.4
10	4.3	7.0	8.5	14.6
5	3.7	5.8	7.1	11.3
2	4.0	6.5	6.6	15.3
1	3.9	6.0	7.6	—

*Cells incubated in THC at indicated concentration, washed twice in medium to remove excess THC and tested for cytotoxicity at the times indicated.

†Mean percent cytotoxicity $= \frac{\text{counts/min experimental}}{\text{counts/min maximum release}} \times 100$

measured by ^{51}Cr release from three separate cultures using 2×10^6 cells/well. Variation between replicates was always less than 5% of the counts/min. Representative experiment from one patient.

‡Concentration of DMSO equivalent to that used to dilute THC to 20 μg/ml.

Table 2. Suppression of natural killer cell activity by THC as function of time of exposure

Addition to cultures	Percent specific cytotoxicity after incubation with THC for* 18 h	4 h	3 h	2 h	1 h	0 h
Control (medium)	31.0	43.0	32.5	57.3	48.5	54.0
DMSO (0.1%)†	36.0	40.6	33.0	47.5	46.4	39.7
	(116.3)‡	(94.4)	(101.5)	(82.9)	(95.7)	(73.5)
THC (20 μg)	—	0.6	0.5	4.0	4.3	55.3
		(1.4)	(1.5)	(7.0)	(8.9)	(102.4)
(10 μg)	0.6	15.6	8.6	26.4	28.17	47.4
	(1.8)	(36.3)	(26.5)	(46.1)	(59.2)	(87.8)
(1 μg)	—	41.7	35.5	52.3	45.2	50.3
		(97.0)	(109.2)	(91.3)	(93.2)	(93.1)

*Cells incubated in THC at times indicated, washed twice in medium to remove excess THC and tested in 4 h NK assay against K562 target cells.

†Concentration of DMSO equivalent to that used to dilute THC to 20 μg/ml.

‡Percent of control.

PBLs separated by Ficoll–Hypaque centrifugation for use in the NK assay. Cells were adjusted to 1.1×10^6/ml RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics and distributed into 12 × 75 mm plastic tubes (0.9 ml per tube).

THC treatment

Tetrahydrocannabinol (THC) was obtained from the National Institute on Drug Abuse, Research Technology Branch, Rockville, MD and reconstituted at 20 mg per ml in dimethyl sulfoxide (DMSO). This stock was diluted in RPMI 1640 medium immediately prior to addition to cultures. DMSO was also diluted in a similar RPMI 1640 and included as controls. THC was added to tubes in 0.1 ml amounts to yield final concentrations of 20, 10, 5, 2 and 1 μg/ml. Control cultures were incubated in either medium only or DMSO diluted in medium to a final concentration of 0.1%. Cells were incubated with THC for various times, ranging from 1 to 18 h at 37°C, in a humified atmosphere of 5% CO_2, 95%

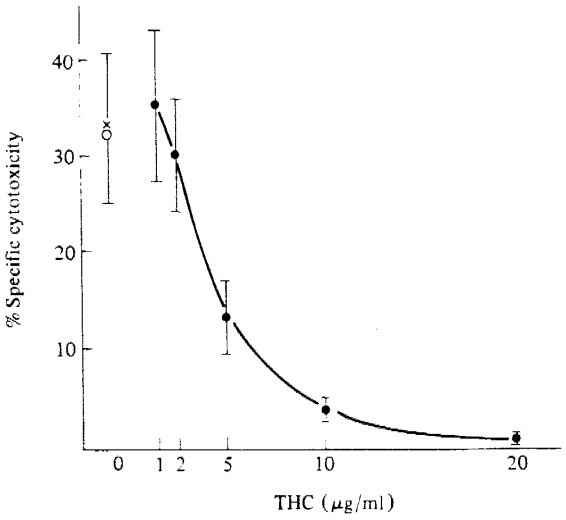

Fig. 1. Effect of THC on human natural killer cell activity. Effector cells were incubated in increasing concentration of THC (●), DMSO (○) or RPMI 1640 medium (X) for 3 h, then washed and incubated with 5 × 10^3 target cells at a ratio 50:1. Each point represents the mean of seven experiments ± S.E.M.

air. After incubation, cells were suspended in 5 ml RPMI 1640 to dilute the THC; then centrifuged at 500 **g** for 10 min at 4°C. The supernatant fluid was removed, the pellet was resuspended in 5 ml RPMI 1640 and centrifuged again to remove unbound THC. Supernatant fluid was again removed and cells were resuspended in RPMI 1640 and adjusted to 2.5 × 10^6 viable cells/ml, as assessed by trypan blue dye exclusion.

Natural killer cell assay

Target cells were the NK sensitive K562 erythroleukemia cell line which is commonly used to assess human NK activity. Cells were labelled with chromium-51 (Na_2 $^{51}CrO_4$) as described previously (Moody, Specter, Bendinelli & Friedman, 1984). Cells were washed free of execess chromium and adjusted to 5 × 10^4/ml RPMI 1640. 0.1 ml was added to wells of a 96 well round bottom microtiter plate for the assay. Effector cells were added to wells in 0.1 ml medium so that 2.5 × 10^6 cells/ml yielded an effector: target ratio of 50:1. The microtiter plate received also target cells only or target cells plus 0.1 ml medium. The former were used to measure maximum release and the latter spontaneous release. The plates were centrifuged at 250 **g** for 2 min and then incubated for 4 h at 37°C. Maximum release was attained by lysing target cells with 0.1 ml 10% sodium dodecyl sulfate, mixing well and removing 0.1 ml of the mixture. The remaining cells in the plates were then centrifuged at 500 **g** for 10 min and 0.1 ml supernatant fluid removed and placed in 7 × 35 mm glass tubes for counting in a gamma counter (United Technologies, Downers Grove, IL). Specific cytotoxicity was calculated using the formula

$$\% \text{ specific cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}}.$$

Toxicity of the THC for lymphocytes was measured using lymphocytes labelled with ^{51}Cr. As can be seen in Table 1, when cultured for up to 3 h in THC lymphocytes were killed by the 20 μg/ml dose but not lower doses ranging from 10 to 1 μg/ml. The DMSO control was not substantially different from leukocytes incubated in culture medium alone. Visual counting for viability using trypan blue dye exclusion confirmed these results. On the basis of these findings cells were always readjusted after incubation in THC so that cell counts reflected the number of viable cells at the time of initiation of the NK assay.

RESULTS

Pretreatment of leukocytes with THC in doses ranging from 1 to 20 μg/ml for up to 18 h revealed that there was a direct relationship between the time of exposure and the resulting suppression of NK activity (Table 2). Virtually all NK activity was lost upon exposure to 20 μg/ml THC for 3 h and 10 μg/ml for 18 h. Incubation for 1 or 2 h resulted in proportionately less suppression, whereas addition of THC directly into the 4 h NK assay (time 0) had no appreciable effect even at 20 μg/ml.

Additional experiments were performed using a pre-incubation of 3 h in THC, then removal of the drug for the NK assay. As can be seen in Fig. 1, as little at 5 μg/ml THC had a marked suppressive effect on NK function. However, 2 or 1 μg/ml had no noticeable effect. Suppression was highly dose dependent with a stronger effect noted as the dose increased to 20 μg/ml. The NK depressive effects of THC were observed over a broad range of effector to target cell ratios. However, examination of percent reduction of cytotoxicity vs positive controls revealed that a consistent suppressive effect of THC at 10 and 20 μg/ml is achieved at all E:T ratios (Table 3).

Table 3. Effect of THC on natural killer cell activity at difference effector to target cell ratios

Additions to cultures	Percent specific cytoxicity 50:1*	25:1	12:1	6:1	3:1
Control (medium)	49.1†	38.8	22.2	12.1	7.0
DMSO (0.1%)	65.8	47.1	31.2	17.3	10.3
	(134.0)‡	(121.4)	(140.5)	(144.2)	(147.1)
THC (20 μg)	0.9	0.0	0.8	0.2	0.0
	(1.8)	(0)	(3.6)	(0.2)	(0)
(10 μg)	9.8	5.0	3.4	2.8	1.3
	(20.0)	(12.9)	(15.3)	(12.2)	(18.6)
(1 μg)	53.0	38.4	23.9	12.8	7.9
	(107.9)	(99.0)	(107.7)	(106.7)	(112.9)

*Effector:target.
†Mean of two individuals.
‡Percent of control.

DISCUSSION

The data presented demonstrate that delta 9-THC, when added to human peripheral blood leukocytes, decreases their ability to function in the 4 h NK cell cytotoxicity assay. This activity was dependent upon the length of time the effector cells were exposed to THC and the dose of THC. Killing was independent of the effector to target cell ratio over a wide range. Although THC was cytotoxic for PBL at a dose of 20 μg/ml, this cannot be the explanation for the suppressive effects of the drug since cells were readjusted at initiation of the NK assays. Lower doses, i.e. 10 μg/ml and 5 μg/ml, were also highly suppressive for NK cell function but did not have any effect on effector cell viability.

The mechanism by which THC is capable of suppressing NK activity is as yet unclear. Preliminary experiments to delineate this mechanism have shown that indomethacin has no effect in reversing this suppression (unpublished data). Thus suppression cannot be attributed to induction of prostaglandins, since indomethacin is known to inhibit prostaglandin synthesis (Brunda *et al.*, 1980). This is an important observation since prostaglandins have been shown to suppress NK function in other systems (Brunda *et al.*, 1980; Droller *et al.*, 1978; Kendall & Targan, 1980). It is also possible that the lipophilic nature of THC results in an interference with ion channels in the NK cell membrane. Recently, a preliminary report has appeared (Schlichter & Sidell, 1985) demonstrating a suppression of NK killing by potassium and calcium channel blockers. This reduced NK activity in THC exposed PBL may be a highly significant observation since THC has been implicated, in experimental infection models, in enhancement of susceptibility to infection (Bradley, 1984; Juel-Jenson, 1972; Morahan, Klykken, Smith, Harris & Munson, 1979). Such enhanced susceptibility could be due to suppressed NK activity.

Interestingly, other psychoactive agents have recently been reported to alter NK cell function. Two separate reports indicate that either endorphins (Kay, Allen & Morley, 1984) or enkephalin (Faith, Liang, Mungo & Plotnikoff, 1984) can enhance NK activity. Conversely, morphine and "opioid stress" resulted in depression of NK cytotoxicity (Shavit, Lewis, Terman, Grale & Liebeskind, 1984). While these data do not allow any generalization about psychoactive agents and NK activity, they do lead to speculation about possible mechanisms for the immunomodulatory activities. Suppression of NK function could be generated indirectly by THC by stimulating the release of immunosuppressive hormones like adrenocoritcosteroids, which are known to inhibit NK activity (Cox, Holbrook, Grasso, Specter & Friedman, 1982; Hochman & Cudkowitz, 1979); or by inhibition of interferon synthesis. Morphine, for example is known to depress interferon levels (Hung, Lefkowitz & Geber, 1973). While the mechanism of THC induced suppression remains to be determined, the results generated by other psychoactive agents have yielded clues as to how this may be pursued.

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