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DETECTION OF Δ⁹-TETRAHYDROCANNABINOL IN SALIVA OF MEN BY MEANS OF THIN-LAYER CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

The saliva of men is suggested as a useful biological material for revealing cannabis abuse. Ten subjects volunteered and smoked a single tobacco cigarette that contained an amount of cannabis equivalent to 2.8 mg of Δ⁹-tetrahydrocannabinol. After smoking, the Δ⁹-tetrahydrocannabinol was detected in the saliva extracts by means of two-dimensional thin-layer chromatography and mass spectrometry for periods up to 2 h.

INTRODUCTION

Basic knowledge about the human metabolism of Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the major psychoactive principle in marihuana[1], was obtained by experiments with [¹⁴C]Δ⁹-THC (refs. 2–4). These experiments showed, for example, the rapid decline of the concentration of Δ⁹-THC in plasma within the first 2 h after cannabis intake or the lack of excretion of non-metabolized Δ⁹-THC into the urine. By measuring the variation of the concentration of Δ⁹-THC in plasma with time by means of combined gas chromatography and mass spectrometry, results that corresponded well with the results of radioactivity experiments were obtained[5].

Recently, the saliva was suggested as a biological material that could be used in order to reveal cannabis abuse[6]. Δ⁹-THC was detected fluorimetrically by reaction with 5-dimethylaminonaphthalene-1-sulphonyl chloride (Dns-Cl)[6,7]. Fluorescent labelling of cannabinoids with Dns-Cl, which was also investigated by Forrest and coworkers[8,9] and was discussed as a possible means for forensic use, lowered the detection limit to about $1 \cdot 10^{-12}$ mole. For the detection and identification of Δ⁹-THC in the saliva of men who have smoked a marihuana cigarette, this method was found to be very useful. It was indicated that both contamination of the mouth cavity caused by smoking the marihuana cigarette and excretion of the Δ⁹-THC in the saliva by the salivary glands[6] would be possible mechanisms for the detection of Δ⁹-THC in the saliva. This assumption was recently confirmed by autoradiographic experiments on

the monkey[10]. Thirty minutes after the intravenous injection of [2,4-^{14}C]-Δ^9-THC, the secretory cells around the ducts of the salivary glands appeared heavily labelled and THC was detected in the saliva[11].

This paper now demonstrates the possibility of detecting Δ^9-THC in the saliva of men by means of thin-layer chromatography (TLC) and mass spectrometry (MS).

MATERIALS AND METHODS

For the experiments, ten student volunteers (six male and four female) were used. All of the subjects had smoked cannabis once before but were not permanent cannabis users. All of the subjects, with one exception, were tobacco-cigarette smokers. They were instructed before the experiments to inhale the smoke of the cannabis cigarette deeply, but this instruction was only partially followed. Each subject smoked one cigarette containing 700 mg of cannabis (0.4% Δ^9-THC content) corresponding to 2.8 mg of Δ^9-THC. The smoking was not standardized in any form but was mostly completed within 10 min.

Extraction of saliva and blood

Volumes of 3 ml of saliva were collected before smoking and at 5, 15, 30, 60, 90 and 120 min after finishing smoking, in 25-ml glass-stoppered centrifuge tubes. Blood was taken in amounts of 2–3 ml from the arm veins of the subjects before smoking and 1 h after the smoking was completed. Care was taken that the smoking of the cannabis cigarette and the collection of the biological material were carried out in different rooms.

The saliva and blood were extracted three times using 5-ml portions of light petroleum (b.p. 40–60)–methyl acetate (2:1). The extracts were dried for 1 h by adding small amounts of sodium sulphate. After drying the extracts, the sodium sulphate was removed and the solvent was evaporated under a stream of nitrogen. All of the solvents employed were purified thoroughly and the grade of purity was confirmed by MS.

Thin-layer chromatography

Glass sheets of dimensions 20 × 20 cm were carefully cleaned and coated with a 0.2-mm layer of silica gel G (Merck, Darmstadt, G.F.R.). The sheets were first air-dried, then activated at 100 and stored in a desiccator over calcium chloride.

For two-dimensional TLC, the extracted residues were re-dissolved in light petroleum (b.p. 40–60). A reference sample of Δ^9-THC was used in each TLC run, in both dimensions. The solvent systems used for developing the saliva extracts were *n*-heptane–acetone (90:10) for the first dimension and cyclohexane–benzene–methanol –ethyl acetate (70:30:1:1) for the second dimension. The chromatograms were developed by two runs in the first dimension and one run in the second dimension.

TLC of the blood extracts was carried out using the solvent systems light petroleum (b.p. 40–60)–diethyl ether–acetic acid (40:10:1) and *n*-heptane–diethyl ether (80:20) for the first and second dimensions, respectively. The chromatograms were developed by two runs in both the first and second dimensions. The chromatographic tanks were coated with filter-paper and four chromatograms were developed simultaneously in one tank. The grade of purity of silica gel and of all of the solvents

employed was examined by MS. For detecting the Δ⁹-THC reference samples, each chromatogram was partially sprayed with fast blue salt B (Merck) dissolved in 0.1 *N* sodium hydroxide solution.

Elution of Δ⁹-THC. The zones corresponding to the Δ⁹-THC reference samples were circled on each chromatogram and, as usual, the silica gel was quantitatively removed using a small capillary under a slight vacuum. A small portion of cyclohexane–benzene (100:0.5) was sucked through the glass capillary containing the silica gel and Δ⁹-THC was then eluted using ethyl acetate. The ethyl acetate solution was easily transferred into a small self-prepared glass tube for MS.

Mass spectrometry

Low-resolution mass spectra were obtained using a Varian CH-5 mass spectrometer (direct input; 70 eV; 150–160°).

RESULTS AND DISCUSSION

After a subject had smoked a cigarette containing 10 mg of Δ⁹-THC, which is a relatively high dose, the concentration of Δ⁹-THC in the plasma decreased from about 20 pmole·ml^{-1} 10 min after smoking to about 7 and 2 pmole·ml^{-1} 1 h and 2 h after smoking, respectively[1,5]. Hence concentrations of Δ⁹-THC in plasma of about 2 and 0.5–1.0 pmole·ml^{-1} should be reached 1 h and 2 h, respectively, after smoking a cannabis cigarette containing 2.8 mg of Δ⁹-THC. These concentrations are almost outside the detection limit of most analytical techniques. In subjective statements, the volunteers revealed that 2.8 mg of Δ⁹-THC per cigarette was a low dose. Most of the subjects reported that they felt only slightly "high" compared with former experiences with the drug.

In Fig. 1, a typical two-dimensional thin-layer chromatogram of a saliva ex-

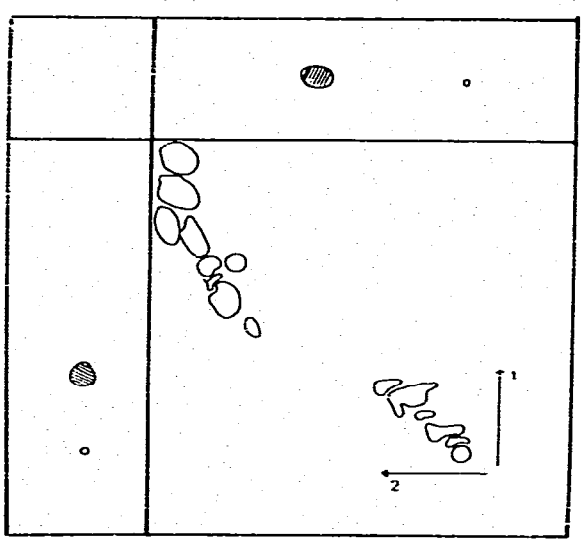

Fig. 1. Typical pattern of a saliva extract after two-dimensional TLC and spraying with phosphomolybdic acid. Δ⁹-THC is layered on the chromatogram as a reference for both dimensions.

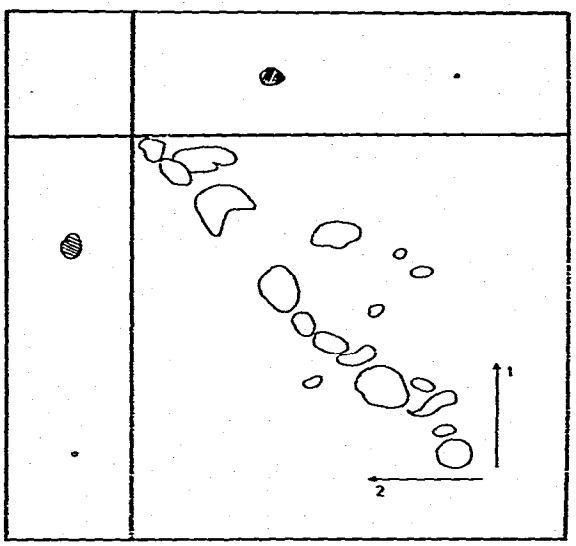

Fig. 2. Typical pattern of a blood extract after two-dimensional TLC and spraying with phosphomolybdic acid.

tract is shown, while Fig. 2 represents the two-dimensional thin-layer chromatogram of a blood extract. It can be seen that TLC was performed in such a way that no overlapping of the Δ^9-THC zones occurred with the larger concentrations of substances normally present in saliva and blood. Parts of the mass spectra obtained after the elution of the Δ^9-THC zones after TLC of saliva extracts of two subjects before the smoking of a cannabis cigarette are shown in Fig. 3A and 3B. The analysis of saliva extracts of at least 20 different persons who had not been in contact with marihuana gave mass spectra with different fragmentation patterns. Often, but not in every case, an unidentified ion with *m/e* 284 appeared with different intensities. Peaks corre-

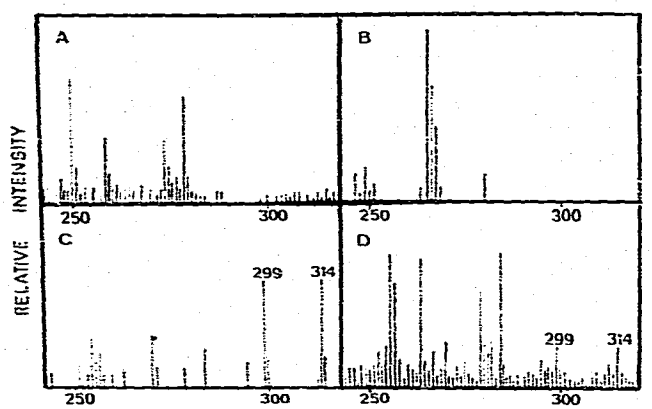

Fig. 3. A and B show parts of the mass spectra of the saliva extracts of two subjects before smoking a cannabis cigarette. C and D show parts of the mass spectra of the saliva extracts of two subjects 1 h and 2 h after smoking a cannabis cigarette. The mass spectra were obtained by eluting the Δ^9-THC zones after two-dimensional TLC.

sponding to ions with higher *m/e* values were also sometimes recorded. In no case were ions with *m/e* 299 and 314 observed in these spectra.

Fig. 3C and 3D show parts of two mass spectra obtained after TLC of saliva extracts carried out 1 h and 2 h after smoking. In both spectra the molecular ion *m/e* 314 and the fragment with *m/e* 299 formed by splitting off a methyl group were recorded. In all experiments, Δ^9-THC could be detected in the saliva of the subjects within the first 30 min after smoking. At 60 and 90 min after the smoking the saliva extracts showed the presence of Δ^9-THC in 90% and 80% of the samples, respectively. Δ^9-THC could still be detected in 50% of the investigated saliva extracts 2 h after the smoking (Fig. 4). On the other hand, Δ^9-THC could be found in only two blood samples. When Δ^9-THC was detectable in the blood, it was also detectable in the saliva 2 h after the smoking.

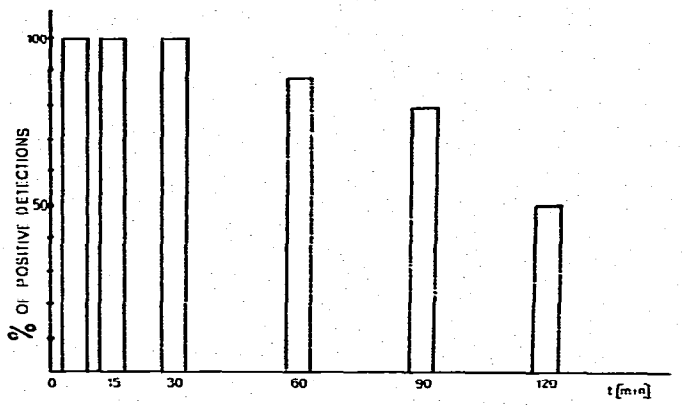

Fig. 4. Possibility of detecting Δ^9-THC in saliva of men after smoking a cannabis cigarette containing 700 mg of cannabis, corresponding to 2.8 mg of Δ^9-THC. Ten subjects volunteered for the experiments.

In 30 mass spectra of Δ^9-THC, all obtained under the conditions mentioned above, the ratio of the intensities of the ions *m/e* 299 and 314 were determined. In 29 spectra, this ratio varied between 0.9 and 1.1, while in one spectrum it was 0.8. The ratio of the peak intensities can be an additional valuable indication of the presence of Δ^9-THC. By adjusting the mass spectrometer to record only the intensities of the ions *m/e* 314 and 299 it should be possible to improve the Δ^9-THC detection limit.

The excretion of Δ^9-THC by the salivary glands after the intravenous application of the drug was demonstrated in the monkey but it was not investigated whether this also occurs in man. When the cannabis cigarettes were smoked completely without inhalation of the smoke, extremely low concentrations of Δ^9-THC may reach the salivary glands. However, owing to contamination of the mouth cavity, Δ^9-THC was detectable in the saliva within a period of 1 h after the smoking.

In the above experiments, where the ten subjects were instructed to inhale the smoke of the cannabis cigarettes, the detection of Δ^9-THC was still possible 2 h after the smoking. These results suggest that excretion of Δ^9-THC by the salivary glands may particularly contribute to the possible Δ^9-THC detection after periods longer than 1 h after the smoking.

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