Neuroprotection by Tetrahydrocannabinol in an *in vivo* excitotoxicity-model as studied by Diffusion-Weighted and T_2-Weighted MRI

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Introduction

The active component of marijuana, tetrahydrocannabinol (THC), has been used for its medicinal and psychoactive properties for more than 2000 years. Recently, several *in vitro* studies have been reported in which THC exerted neuroprotective properties in neurons subjected to excitotoxicity, which is a model for ischemia and various neurodegenerative diseases such as Huntington's and Parkinson's disease [1,2]. However, it is still unknown whether THC affords neuroprotection in an *in vivo* model of excitotoxicity. We recently developed an *in vivo* model of neuronal injury in the neonatal rat in which ouabain, a Na^+/K^+-ATPase inhibitor, is used to elicit neuronal death. Here, we present the first results of a MRI-study in which we tested the neuroprotective properties of THC in this model.

Materials and Methods

Animal model

Neuronal injury is induced in ether-anesthetized neonatal Wistar rats (PND7-8) by unilateral intrastriatal injection of ouabain (0.5 nmol in 0.5 µl, n = 6) or vehicle (0.5 µl 40 mM Tris-HCl buffer, pH 7.4, n=1) at a rate of 0.125 µl/min using a microdrive. Animals were then positioned in the magnet and anesthesia was continued using a mixture of halothane (0.4-1%) in $N_20/0_2$. Body temperature was maintained at 37°C. Three rats received also an *i.p.* injection of THC (1 mg/kg in PBS/Tween80/ethanol (18:1:1 v/v)) 30 min prior to excitoxin-injection.

MR-protocol

MR-experiments were performed on a 4.7T horizontal bore spectrometer with a 220 mT/m gradient insert. Radiofrequency excitation and signal detection were accomplished by means of a Helmholtz volume coil (9 cm diameter) and a surface coil (2 cm diameter), respectively. A multislice, single-shot diffusion trace MRI-technique was used in order to generate quantified images of tissue water trace apparent diffusion coefficient (ADC) (double spin echo sequence, 7 1.5 mm slices, 64 x 64 data matrix in a 2.2 x 2.2 field of view, 4 *b* values (100-1300 s/mm^2)). Multislice T_2-weighted images were measured using a spin-echo sequence, with four different echo times (18, 40, 62 and 84 ms). DWtrace and T_2W-MRI was performed after excitotoxin-injection and also after 7 days.

Data-analysis

ADC and T_2 maps were generated by mono-exponential fitting using IDL. Parametric images were analyzed in anatomic regions of interest with the use of ImageBrowser (Varian). Brain tissue in an ipsilateral pixel was considered pathological when its signal intensity differed more than 2x the standard deviation of the mean intensity of the pixels in the contralateral hemisphere.

Results

In diffusion-weighted images cytotoxic edema, indicated by hyperintense regions, was observed within 15 min of ouabain injection in both non-treated and THC-treated rats, whereas in the control animal no changes were noted. Different brain areas such as the parietal cortex and the caudate putamen were affected by ouabain. ADC-values calculated in the ipsilateral hemisphere were in both groups reduced with 42% to $0.69x10^{-3}$ mm^2/s compared to the contralateral hemisphere ($1.20x\ 10^{-3}$ mm^2/s). The volume of affected tissue was 9% smaller in THC-treated rats (Table 1). After seven days hyperintensities were found in ADC-maps indicating the presence of vasogenic edema and tissue loss. Compared to the contralateral ADC-value ($1.06x10^{-3}$ mm^2/s), the ipsilateral ADC-values were increased to $1.64x10^{-3}$ mm^2/s (+55%) and $1.52x10^{-3}$ mm^2/s (+43%) for non- and THC-treated rats, respectively. At this time point the infarct volume, based on these hyperintensities, was increased by 19% in the non-treated group, whereas the infarct did not grow in animals which received THC. Ultimately, this resulted in a 27% smaller infarct volume in THC-treated rats.

Acutely after ouabain injection no differences in T_2-values in the ipsilateral hemisphere were observed in T_2-weighted maps compared to the contralateral side for all rats. After a week hyper- and hypointense regions were found in the ipsilateral side (Fig. 1). The T_2-relaxation times were increased with 69% to 125 ms in the hyperintense regions compared to a T_2 of 73 ms in the contralateral hemisphere. T_2-values in hypointense areas were decreased with 25% to 55 ms. A reduction of 29% in infarct size, based on both hypo- and hyperintensities, was observed in T_2-maps for THC-treated rats (Fig.2). Tissue loss and vasogenic edema, as indicated by hyperintensities, was reduced with 36%, while the overall volume of brain tissue affected by astrogliosis, based on hypointensities, was 21% smaller. In the parietal cortex a reduction in hypointense tissue of even 43% was observed.

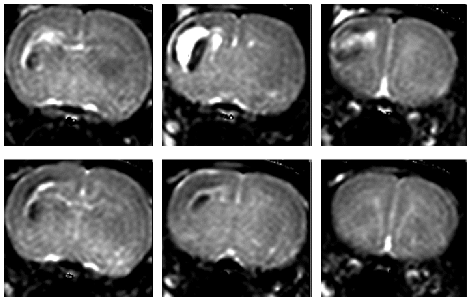

Fig. 1 Three adjacent coronal parametric images of tissue water T_2 relaxation time in neonatal rat brain as measured in 7-day-old pups. Upper row: ouabain injected control. Lower row: animal treated *i.p.* with THC (1 mg/kg).

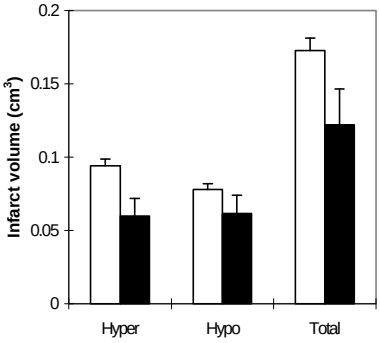

Fig. 2 Infarct size in neonatal rat brain as measured 7 days after intrastriatal ouabain injection based on T_2-maps. Error bars represent S.E.M. (white bar = non-treated, black bar = THC treated).

Table 1: Infarct volumes based on ADC-maps.

THC	Volume day 0 ± S.E.M. (cm^3)	Volume day 7 ± S.E.M. (cm^3)
-	0.053 ± 0.001	0.063 ± 0.008
+	0.048 ± 0.005	0.050 ± 0.013

Discussion

These data are the first to suggest that THC can indeed reduce neuronal damage in an *in vivo* model of excitotoxicity. As yet, the mechanism underlying the *in vivo* neuroprotection has to be established. Several ways of action are likely to contribute to the neuroprotective properties of THC, including: *i*) inhibition of Ca^{2+}-channels via the activation of CB1 receptors, thereby preventing glutamate release and activation of destructive cascades; *ii*) its anti-oxidative properties leading to scavenging reactive oxygen species; and *iii*) its anti-inflammatory actions via activation of the CB2 receptor.

References [1] Hampson et al., 1998, Proc. Natl. Acad. Sci. USA, 95, 8268-8273; [2] Shen and Thayer, 1998, Mol. Pharmacol., 54, 459-462