Diffusion Weighted Imaging in a Rat Closed Head Injury model gives new insights in the understanding of the neuroprotective effect of 2-farnesyl thiosalicylic acid, FTS, a synthetic Ras inhibitor

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Introduction: Cerebral blood flow (CBF) impairment is a main factor contributing to morbidity and mortality after traumatic brain injury (TBI). TBI activates cellular and molecular responses resulting in secondary damages leading to 1. PTKs and MAPK induced decrease in vasodilatation through K-channel inactivation[1] 2. neuroinflammation as a result of PKC or NOS mediated free radical productions (OH., O2.- and ONOO-) and 3. neuronal cell death. Inhibition of the neuroinflammatory response, maintenance of the cerebral perfusion pressure and restoration of the compensatory vasodilatory and vasoconstrictory properties of the cerebral vasculature are critical for the effective treatment of injured patients[2] . The involvement of Ras-GTP in those processes was well established. For this reason, FTS, a synthetic Ras inhibitor[3], was tested as a neuroprotective agent in a TBI rat model. The pharmacological results obtained encouraged us to undergo MRI measurements in order to test if this therapeutic effect was measurable in-vivo and also to get some physiological insight. The MRI protocols include diffusion weighted imaging from which apparent diffusion coefficients (ADC) maps and semi-quantitative perfusion maps were compiled, and anatomical images from which T2 maps were compiled. The increase in T2, the nuclear magnetic resonance transverse relaxation time of water, after trauma is usually attributed to vasogenic edema and infracted areas. The ADC is sensitive to the cytotoxic edema defined as the relative change in the volumes of extracellular versus intracellular spaces triggered by energy failure. MRI distinguishes between the water flowing in capillaries (perfusion), and static water (pure diffusion of intra and extracellular water)[4].

Animals and trauma. TBI was induced under ether anesthesia on adult male Sabra rats ~250 g each, followed by supporting oxygenation with 100% O2 for ~ 2 min. Two groups were studied: 1) an untreated group (n=6) for which only vehicle was injected after TBI 2) a FTS treated group (n=6). FTS was injected 5 mg/kg i.p one hour after TBI. A neurological severity score (NSS) was assessed at 1 hour after TBI to insure that the damage was similar for all the animals. The MRI recording was performed on the rats under anesthesia (isoflurane/N_2O/O_2) for a period of ~ 3 hours, before TBI in order to acquire pre-TBI baseline data, then 1.5 hour after TBI. The MRI protocol was repeated at 24 hours and at 1 week following TBI.

Magnetic Resonance Imaging (MRI) measurements were performed on a 4.7T BioSpec system (Bruker) equipped with active shielded gradients (Gmax=72mT/m). Radio frequency pulses were transmitted using a 20mm transmitter/receiver surface coil placed over the skull and centered over the rat midline. Diffusion axial images were recorded with a spin echo sequence (TR= 2000msec, TE=48.2 msec, matrix size: 128*128, FOV=3cm, 4 averages, seven axial brain slices , slice thickness: 1.2 mm and interslice distance: 1.6mm, 4 b values: 9.3, 312.6, 917.4, 1340.3 sec/mm2, Δ=40msec, Gr=39.1467mT/m, δ=3 msec; Each data set consisted of 28 images. Anatomical axial images (TR=2000ms, TE=45ms, matrix size: 256*256, 4 averages) were acquired as well. Four different TE were used (45, 90, 135 and 180ms) to generate T2 maps. A built-in automatic program of Paravision calculated the ADC maps by fitting a mono exponential decay of the signal for each pixel6. ADC maps, T2 maps, pure Diffusion maps and Perfusion maps and anatomical images were displayed and the ADC(sec/mm2) and T2 (msec) values retrieved, using IDL (Interactive Data Language). Data analysis. The mean ADC values were calculated for 12 anatomically defined regions of interest (ROI). The data matrix contains the ADC mean values for 12 ROIs in 6 slices for 12 rats at 4 time points (Baseline; the day of CBI; 24 hours after CBI and 1 week after CBI). The percent of changes from baseline were calculated to compare any set of data and averaged for each anatomical region (Striatum-a4, thalamus-a5, hippocampus-a6, cingulated-a2, dorsolateral-a3 and occipital cortex-a1), leading to a smaller matrix 12 anatomical areas x 12 rats x 3 time points.

Results: The results analysis shows that there is a measurable therapeutic FTS effect in the brain of rats after TBI: From the day following TBI to one week later, ADC values increase as compared with baseline. In contrast, untreated rats exhibit a pronounced decrease of ADC values. One week after TBI, T2 values of FTS rat brains return to baseline while in the untreated rats they are still high. In FTS rats, the perfusion contributes 51% (1 day) and 26% (1 week) to the increase in ADC values. In untreated rats, the decrease of the perfusion contributes 57% (1 day) and 79% (1 week) to the decrease of the ADC values. (Two graphs below.)

Conclusion: From these results, it seems that FTS rescues the brain after TBI by increasing the cerebral perfusion and diffusion This effect can be attributed to a K-channel mediated increase in vasodilation beside the assumed decrease in Ras-dependent MAPK mediated neuroinflammatory response to TBI.

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