MRI evaluation of morphological changes following soman exposure and galantamine treatment

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Introduction: Organophosphorus compounds (OPS) are a class of highly toxic substances that include a number of pesticides as well as the nerve agents soman, sarin, tabun and VX. Nerve agents are feared to be the most lethal chemical weapons ever developed. The acute toxicity of nerve agents and of OP insecticides has been associated with their potency to irreversibly inhibit acetylcholinesterase (AChE). The limitations of countermeasures against OP poisoning are well recognized, and more effective antidotes have been sought. We have previously demonstrated that galantamine, a drug used to treat patients with mild-to-moderate Alzheimer's disease [Maelicke and Albuquerque, 1996], effectively counteracts the lethality of multiple lethal doses of the nerve agents soman and sarin in guinea pigs [Albuquerque et al., 2006], the best non-primate model to predict the effectiveness of antidotal therapies against OP toxicity. In this study we used a clinical 3.0 Tesla MRI scanner to examine brain morphological changes induced by a single challenge of guinea pigs with soman and to determine the extent to which galantamine prevents the effects of the nerve agent in the brain.

Materials & Methods:

Animal Preparation: Male and female guinea pigs (35-45 days old) were treated as follows: (i) soman (28 µg/kg, 0.5 ml/kg, sc, i.e. 1.25xLD50 for female or 1xLD50 for male guinea pigs) followed 1 min later by atropine methylnitrate (10 mg/kg, im) or (ii) galantamine (8 mg/kg, im) followed 30 min later by soman and atropine methylnitrate (10 mg/kg, im). All galantamine-treated animals survived the challenge with soman and showed no signs of toxicity. Another group of 6 female guinea pigs (35-45 days old) were treated with a higher dose of soman (1.43xLD50 or 40 µg/kg, 0.5 ml/kg, sc) followed by atropine methylnitrate as above. Animals were anaesthetized prior to each MRI experiment with examine (38.5mg/kg, ip) and acepromazine (1.2 mg/kg, ip). At one hour after the completion of imaging, animals were monitored until full recovery from anesthesia before they were returned to the animal facility. All imaging was performed at four time points, prior to soman treatment (time point A), 1-3 hours following soman exposure (time point B), 24 hours following soman exposure (time point C), and 7 days following exposure (time point D).

Magnetic Resonance Imaging: All MR images were obtained using a 3.0 Tesla Siemens Tim-Trio system equipped with state-of-the-art gradients and 18 channels of RF reception. A standard quadrature wrist coil was used in all the experiments. Animals were placed in prone position with the head of the guinea pig centered within the wrist coil. After obtaining localizer scans, high resolution axial and coronal images were obtained using an inversion recovery pulse sequence with an inversion time of 300 ms and a TE/TR of 15ms/3000ms. T1-weighted MP-RAGE images were also obtained in the axial plane at an in-plane resolution of 390µm x 190µm and a slice thickness of 0.8mm. Acquisition parameters for MP-RAGE were TI/TE/TR of 900ms/2.5ms/1900ms respectively. CPMG pulse sequence was also used to compute the T2-values in various regions of the brain with echo times ranging from 10-120 ms. Images were acquired at an in-plane resolution of 300 µm and a through plane resolution of 1mm. Each animal was imaged four times over seven days.

Image Processing: All guinea pig brains were manually segmented using MIPAV (Medical Image Processing, Analysis, and Visualization http://mipav.cit.nih.gov). Guinea pig brains at all time points were then registered to the first time point (A). One guinea pig brain at time A was identified as a typical brain (target) and all other guinea pig brains at A were registered to this target image. This was repeated for all the other time points. Guinea pigs brains at time B,C, and D were then compared voxel-wise using the paired t-test with the 3D-t-test tool provided within AFNI (Analysis of Functional NeuroImages http://afni.nimh.nih.gov/afni/).

Results: Changes in the piriform cortex, the hippocampus, the olfactory region and the amygdalar regions were visible seven hours after injury. These changes correlated strongly with the animals' cognitive behavior as they exhibited significant trembling, bruxism and convulsions. Segmentation of the brain into gray, white and CSF volumes revealed significant brain atrophy as observed by 27% increase in brain CSF at 30 hours ($p<0.05$). T2- value changes were significantly elevated in the piriform, amygdala and the hippocampus area. While there was some evidence of involvement of the piriform cortex following galantamine treatment (8 mg/kg), no significant morphological changes to the brain or any cognitive abnormalities were observed up to seven days after galantamine treatment.

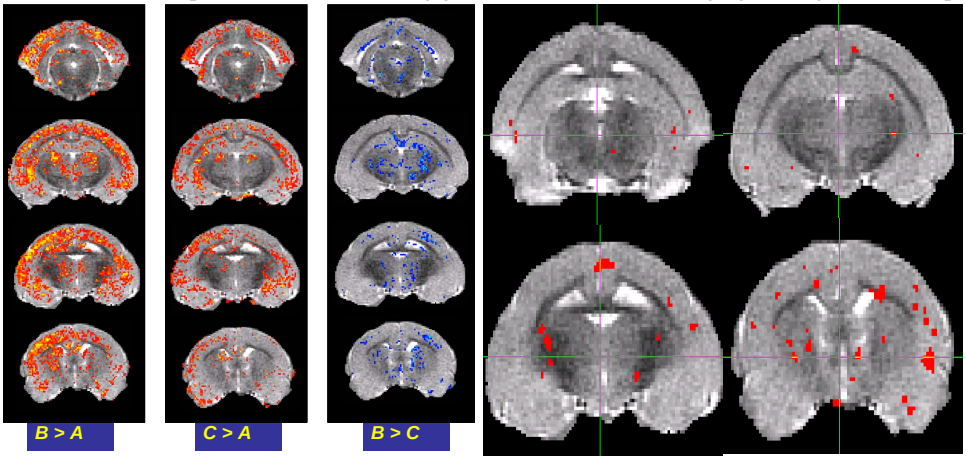

Figure 1. The left panel shows contrast maps of T2-signal intensity changes at different time points following Soman exposure. Maximum changes occur within the first 24 hrs. The right panel shows affected areas following soman exposure and galantamine treatment. Very little changes are seen on these animals.

Conclusions: Morphological changes in the brain can be observed in female and male guinea pigs 1 h after challenge with soman. Significant damage is observed in the piriform cortex, amygdala and hippocampus of guinea pigs following their challenge with soman and the effects are time and dose dependent. Galantamine prevents soman-induced brain damage in female and male prepubertal guinea pigs.

References:

1. Albuquerque et al., PNAS 103:13220-13225, 2006.
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