Perfusion deficit to cholinergic challenge in veterans with Gulf War Illness

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INTRODUCTION: A highly plausible etiology for the newly defined Gulf War Illness (GWI) is that the neural damage and cognitive deficits are associated with excessive exposure to cholinesterase-inhibiting cholinergic stimulants (1-3). This hypothesis is supported by epidemiological findings that many case definitions are linked to cholinergic stimulants such as organophosphate pesticides, pyridostigmine bromide anti-nerve agent medications, and low-level sarin nerve gas. Furthermore, many animal experiments have demonstrated the effect of chemical exposure on brain function, in particular alteration in cholinergic receptors. Our previous SPECT study provided strong indication that cerebral blood flow of veterans with Syndrome 2 GWI has reduced responses to cholinergic challenge, compared to unaffected control veterans (4). However, the SPECT experiment involves injection of radioactive tracer and requires excessively long duration, thereby may not be suitable to large scale study or routinely screening. Therefore, the purposes of the present study are two fold: 1) to use a different CBF technique to confirm the findings from the earlier SPECT study; 2) to establish a cost effective technique that does not require the use of radiotracers and can be routinely performed on clinical MRI systems.

METHODS: A total of 36 male Gulf war veterans were divided into three groups, with 12 (age of 60.9±6.0) in a group identified as Syndrome 2 GWI (characterized by confusion-ataxia), 10 (age of 57.3±6.7) in a group identified as Syndrome 3 GWI (characterized by central pain), and 14 (age of 60.1±6.3) in an age matched control veteran group. Each subject underwent two study sessions 2 days apart, one session for CBF responses to drug infusion (challenge experiment) and the other session for CBF responses to saline infusion (control experiment). At each session subjects were prepared with an intravenous (IV) line for the infusion. The challenge experiment used a short-acting cholinesterase-inhibiting drug physostigmine, which is expected to stimulate the cholinergic system and decrease blood flow. Figure 1 illustrates the timing of the procedures in each session. Baseline perfusion scans without infusion were performed, followed by a brief break period outside the magnet. After the break, the subject was re-positioned and infusion of either saline or physostigmine will be started. For physostigmine, the infusion dose was 1.0 mg. Since our previous pilot study of the dose response showed that the maximal physostigmine effect is achieved by 25 minutes into the infusion and persists for at least 60 minutes more, the infusion perfusion scans were initiated about 25-27 minutes after the onset of infusion. In all subjects, session 1 used saline infusion and session 2 used physostigmine infusion. This order was chosen because some subjects may experience some nausea effects from physostigmine infusion and, if used in session 1, may affect the stress level for session 2. However, the participants were blinded to this order.

All experiments were performed on a Siemens 3T MRI scanner. Arterial-Spin-Labeling (ASL) MRI (FAIR with bolus control) was used to assess regional cerebral blood flow (CBF). The imaging parameters were: bolus width = 800ms; TR=2.8 ms, post bolus delay = 1200ms, 10mm gap on each side of the imaging slab, voxel size 3.48x3.48x3.48 mm^3, 18 slices covering middle portion of the brain, scan duration 8.5 minutes. In addition, we have also measured global CBF in sagittal sinus (in units of ml/min) using a phase-contrast MRI technique with the following parameters: single slice, voxel size = 0.7x0.7x5 mm^3, FOV = 230x230x5 mm^3, maximum velocity encoding = 80 cm/s, scan duration 27 seconds. Although phase-contrast MRI does not provide regional CBF information, it is considered highly quantitative and is complementary to ASL which provides regional information but the CBF quantification is more complex. Paired comparison was performed for the CBF data acquired under saline and physostigmine infusion conditions.

RESULTS and DISCUSSION: With physostigmine challenge, the control group showed a decrease of global CBF, as shown by the phase-contrast results (Fig. 2). This is consistent with the mechanism of physostigmine that it activates the inhibitory brain networks more than the excitatory networks. In contrast, the Syndrome 2 and 3 GWI groups showed unchanged or slightly increased CBF upon physostigmine infusion (Fig. 2). This may be because patients with GWI have lost considerable inhibitory cholinergic receptors and the overall effect of physostigmine becomes excitatory. The differences between the control group and the Syndrome 2 and 3 groups are statistically significant (p=0.05 for control vs Syndrome 2, p=0.02 for control vs Syndrome 3). Regional CBF responses to physostigmine challenge for the three groups are plotted in Fig. 3. Out of the seven deep brain structures covered by the ASL MRI scan, amygdala, hippocampus and caudate showed group level differences. The spatial pattern of the deficit is in good agreement with the previous SPECT finding (4) (Fig. 4). For all of these structures, the Syndrome 2 and 3 groups showed increased CBF. These data suggest that deep brain structures are among the most severely affected regions in patients with Gulf war illness syndrome 2 and 3.

SUMMARY: The present study confirmed and extended previous findings that patients with Gulf War Illness have abnormal response to an inhibitory cholinergic challenge, physostigmine infusion, when compared to age-gender-education matched control veterans. Previous reports were conducted using a radiotracer based SPECT technique and required a total >3 hours for the infusion and brain scan. Our MRI-based technique has several key advantages including shorter experiment duration, complete non-invasiveness and higher spatial and temporal resolutions. This new technique may provide a cost-effective biomarker for characterization of Gulf war illness.

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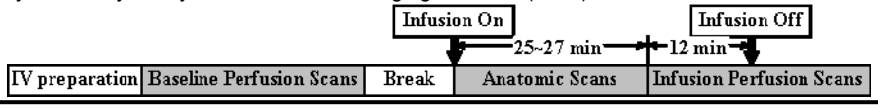

Figure 1: Experimental procedures for the physostigmine challenge.

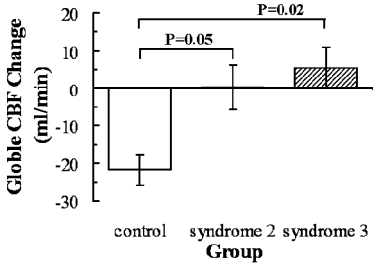

Figure 2: Global CBF change due to cholinergic stimulation measured by phase-contrast MRI.

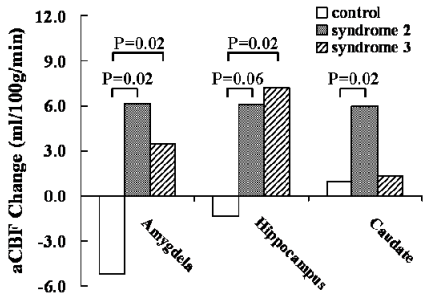

Fig. 3: Regional CBF change due to cholinergic stimulation measured by ASL MRI.

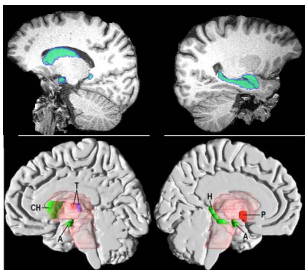

Fig. 4: Brain regions with significant differences measured by standard resolution ASL MRI (upper) and SPECT (lower) in previous study (4).