Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 84 (2006) 207–218
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Multiple vaccine and pyridostigmine interactions: Effects on cognition, muscle function and health outcomes in marmosets ☆

D. Stevens *, E.A.M. Scott, A.P. Bowditch, G.D. Griffiths, P.C. Pearce

Dstl Biomedical Sciences, Porton Down, Salisbury, SP4 0JQ, UK

Received 23 January 2006; received in revised form 19 April 2006; accepted 27 April 2006
Available online 27 June 2006

Abstract

Following active service during the 1990/1991 Gulf Conflict, a number of UK and US veterans presented with a diverse range of symptoms, collectively known as Gulf Veterans Illnesses (GVI). The administration of vaccines and/or the pretreatment against possible nerve agent poisoning, pyridostigmine bromide (PB), given to armed forces personnel during the Gulf Conflict has been implicated as a possible factor in the aetiology of these illnesses. The possibility that long-term health effects may result from the administration of these vaccines (anthrax, pertussis, plague, yellow fever, polio, typhoid, tetanus, hepatitis B, meningococcal meningitis and cholera) and/or PB, have been investigated using a non-human primate model, the common marmoset.

This paper reports the results from three aspects of the study, cognitive behaviour (performance of a touchscreen mediated discrimination task), muscle function (performance of a simple strength test) and general health.

There were no marked long-term changes in cognition, muscle function or health that could be attributed to vaccines and/or PB administration. Statistical differences related to treatments were only observed in two aspects of cognition and one of clinical chemistry. These changes were transient in nature and their magnitude were minor and, in consequence, was not regarded as having long-term biological significance.

Keywords: Vaccines; Pyridostigmine bromide; Non-human primate; Cognition; Muscle function; Health; AChE

1. Introduction

Following active service in the Persian Gulf during the first Gulf Conflict of 1990/1, a number of UK and US veterans presented with a diverse range of symptoms which have become collectively known as Gulf Veterans Illnesses (GVI). A document published by the Parliamentary Office of Science and Technology provides an overview of research relating to these conditions (Border and Norton, 1997), the majority of which have been epidemiological in nature. As well as the complex environment in which they were deployed in the Middle East, UK troops were vaccinated with two anti-biological warfare agent (anti-BWA) vaccines (selected on the basis of contemporary military intelligence), an additional vaccine adjunct and a range of health and hygiene (H&H) vaccines appropriate for deployment to that region. In addition, pyridostigmine bromide (PB) was taken as a pretreatment to help preserve life in the event of nerve agent poisoning.

The study reported here was part of a multi-stage programme of work commissioned by the Veterans Policy Unit Gulf Veterans' Illnesses (VPU GVI) of the Ministry of Defence with advice from an independent, cross-disciplinary panel of experts who have overseen its progress. The work programme was designed specifically to assist in the interpretation of data emerging from the epidemiological studies and addressed the effects of multiple vaccines and PB. This included a mouse study undertaken at the National Institute for Biological Standards and Control (NIBSC) (Rijpkema et al., 1995) and a study of Dstl staff members who were vaccinated against anthrax in the course of their employment. The findings reported here are drawn from a study designed to assess whether administration of the range of vaccines administered to UK military personnel

☆ This study was funded by the Veterans Policy Unit Gulf Veterans' Illnesses (VPU GVI) of the UK Ministry of Defence.
* Corresponding author. Tel.: +44 1980614696; fax: +44 19980613741.
E-mail address: DSTEVENS@dstl.gov.uk (D. Stevens).

0091-3057/$ - see front matter.
doi:10.1016/j.pbb.2006.04.020

during the 1990/91 Gulf conflict, with and without PB, gives rise to long-term adverse effects in a non-human primate model. Results from preparatory phases of this study in guinea pigs and marmosets, which identified dose levels and dose combinations of vaccines and PB that induced measurable responses without producing unacceptable short-term effects, have been reported previously (Griffiths et al., 2001).

The study aimed to investigate whether indices that reflect the most frequently reported signs and symptoms by Gulf veterans, e.g. impaired cognition, sleep disturbances and fatigue (Coker et al., 1999; Unwin et al., 1999; Lee et al., 2001, 2002; Cherry et al., 2001), are observed following administration of multiple vaccines and/or PB. Thus, any changes in physiology, immune response and central function over 18 months following administration could be detected and interpreted and this paper reports the results of cognitive testing, muscle function and general health elements of the marmoset study. The effects on other indices (including sleep, brain electrical activity, pathology and immunology) will be reported in separate publications.

1.1. Cognitive behaviour

Cognitive problems were frequently reported by veterans of the 1990/91 Gulf conflict and these were generally manifested as subjective deficits in concentration ability and memory. The incidence of cognitive dysfunction among Gulf veterans varies between epidemiological studies reported to date. In a questionnaire-based study, Cherry et al. (2001) noted 'frequently' reported cognitive symptoms which occurred twice as often in Gulf veterans than matched controls. Unwin et al. (1999) also found self-reported symptoms occurring at a rate twice that of Bosnia veterans and undeployed Era veterans. No evidence, however, of any major neuropsychological impairment in Gulf veterans was found by David et al. (2002) although Lange et al. (2001) found impairments in attention, concentration and information processing in veterans complaining of fatigue, after taking into account post-war morbidity.

Haley et al. (1997a) suggested 3 syndromes among his study group of 249 veterans, where 175 veterans were complaining of ill health; impaired cognition, characterised by problems with attention, memory and reasoning; confusion-ataxia and central pain. Subsequently, indications of decreased functional neuronal mass in the basal ganglia and brainstem were reported (Haley et al., 2000a). These findings were reported to be associated with altered levels of plasma homovanillic acid and the suggestion was made that 'Gulf War Syndrome' was a neurological illness, in part, related to injury to dopaminergic neurons in the central ganglia (Haley et al., 2000b). Moreover, an NAA/creatine ratio, indicative of hippocampal dysfunction, has also been reported although none of the symptomatic Gulf veterans reported problems with memory or attention (Menon et al., 2004). In other questionnaire based studies of US Gulf veterans, there were 11–33% self-reported cognitive problems compared to 2–11% of military controls (Fukuda et al., 1998; Kang et al., 2000; Murphy et al., 1999; Gray et al., 1999; Proctor et al., 1998).

Other than some assessments of the effects of reversible or irreversible cholinesterase inhibitors, there have been relatively few preclinical studies (e.g. Scremin et al., 2003; Pearce et al., 1999; Muggleton et al., 2005) which have concentrated on effects following recovery to baseline levels of cholinesterase (ChE) levels.

In the present study, an attentional set shifting task, with an additional component to investigate aspects of memory, was used to investigate cognitive changes which could be related to the reported symptoms of "difficulty concentrating" and "forgetfulness" in man. The tests were selected from the Cambridge Automated Neuropsychological Test Automated Battery (CANTAB), a computer based system which has been extensively used in the characterisation of cognitive function in both non-human primates and man. The attentional set shifting test employed was the intradimensional/extradimensional (ID/ED) shift, an analogue of the Wisconsin Card Sort Test (Grant and Berg, 1948). It involves a number of rule changes which have been shown to be differentially sensitive to CNS disruption e.g. brain lesions (Roberts et al., 1992) and pharmacological interventions (Sahakian and Coull, 1993). In order to facilitate long-term repeated presentation of the test, the sequence of stages was adapted from previous human (Sahakian et al., 1990) and non-human primate (Roberts et al., 1988) studies. The home cage approach to testing, which has previously been shown to be practicable (Crofts et al., 1999) and conducive to rapid training and task acquisition (Muggleton et al., 1997), was employed. We have previously used this approach to study the effects of a low dose of sarin (Pearce et al., 1999) and a range of doses of diazinon (Muggleton et al., 2005).

1.2. Muscle function

Muscle weakness has been reported by Gulf veterans, although less frequently reported than attentional deficits. Cherry et al. (2001) found that "feeling too weak to complete what you start" was self-reported twice as often by Gulf veterans than the non-Gulf control group. Amongst 3000 veterans attending the MOD's Gulf Veterans' Medical Assessment Programme, an average of just under 40% reported joint and muscle aches and pains but not weakness per se. There were no objective findings of neuromuscular pathology either in veterans who self-reported muscle weakness, fatigue and myalgia (Amato et al., 1997) or in a comparative study of Gulf veterans and veterans from the Bosnian Conflict (Unwin et al., 1999). It has been hypothesised that some of the symptoms may be related to minor impairments of nerve conduction e.g. carpal tunnel syndrome (Sharief et al., 2002) or mitochondrial insufficiency (Rose et al., 2004) which may not be specifically related to service in the Gulf conflict.

The effects of chronic PB administration in a simulated desert environment were examined by giving healthy soldiers PB for 7 consecutive days (30 mg orally, every 8 h) (Cook et al., 1992). Whilst there was a trend towards decreased grip strength while receiving PB, chronic administration did not negatively impact the soldiers' ability to perform physical work over repeated days in a desert environment. Gray et al. (1999), however, found a significantly lower mean handgrip strength measurement in Gulf veterans who complained of muscle weakness compared to other Gulf veterans.

In vitro studies by Drake-Baumann and Seil (1999) on the effects of PB on neuromuscular junctions have shown that acute