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Lack of Association of Paraoxonase 1 Promoter Polymorphisms with Gulf War Illness

Mike Mackness and Bharti Mackness

Avda. Princip D´Espanya, 43892 Miami Playa, Tarragona, Spain

Corresponding Author: Mike Mackness, Avda. Princip D´Espanya, 43892 Miami Playa, Tarragona, Spain, Tel: +34977170360; E-mail: mike.mackness@gmail.com

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Abstract

Low serum PON1 levels are associated with Gulf War Illness (GWI). We sought to investigate the role of PON1 promoter polymorphisms in defining PON1 levels in GWI.

There were no differences in the distribution of the PON1 -108 or PON1 -909 genotypes between the Gulf War Veterans (GWV) and the controls; however, PON1 activity and concentration were significantly lower in the GWV.

In the control population, PON1 activity was significantly different between the PON1 -108 genotypes in the order CC>TC>TT ($P<0.001$) and in the PON1 -909 genotypes in the order GG>GC>CC ($P<0.001$). However, in the GWV, such activity differences between genotypes were not evident. In the GWV PON1 activity was significantly lower in all the PON1 -108 and -909 genotypes compared to controls.

There were no differences in PON1 concentration between any of the PON1 -108 or -909 genotypes in either population, however, the concentration was lower in all genotypes in the Veterans compared to the controls.

Low serum PON1 levels appear to be related to symptoms of GWI independent of PON1 promoter and coding region polymorphisms. More studies with larger cohorts are required to define a role for PON1 in GWI.

Keywords: Paraoxonase 1; SNPs; Persian Gulf War; Gulf War Illness

Introduction

Organophosphorus compounds (OPs) are widely used in both rural and urban settings leading to widespread exposure. OPs are activated in the body by the process known as oxidative desulphuration to produce the toxic oxon forms. Some but not all parent or activated OPs are substrates for the serum enzyme paraoxonase-1 (PON1), of those that are (which include some of the most widely used OPs including diazinon and chlorpyriphos (CP) oxons), most are hydrolysed at different rates by the PON1-Q and R isoforms. Therefore, the majority of studies in this area have concentrated on PON1 as a genetic determinant of OP toxicity [1].

Animal studies have consistently shown that PON1 protects against OP toxicity. The administration of exogenous PON1 to rats and mice protects against OP toxicity and administration of the PON1 isoform that hydrolyses the OP at the greatest rate affords the most protection [2]. PON1 knock-out mice are dramatically more susceptible to diazoxon and CPoxon toxicity and the administration of exogenous PON1 restores resistance to these OPs [3].

Military personnel deployed in the Persian Gulf War of 1990-91 were exposed to low levels of the OP nerve gas sarin and various OP insecticides as well as other chemical and biological agents [1]. Neurological symptoms in veterans of the Persian Gulf War have been reported to be associated with chemical exposure to such compounds as OPs, DEET and pyridostigmine [4-6], but not with other putative risk factors, such as smoke from oil-well fires, combat stress, immunisations, or the use of depleted uranium in weaponry [7]. Widespread repeated exposure to chemical agents including OP pesticides and nerve gases, the insect repellent DEET and pyridostigmine occurred during the Gulf War. Nevertheless the causes of the illness found in Gulf War Veterans remain controversial.

PON1 activity has been found to be lower in GWV with GWI than in matched controls [8, 9], however, these findings were independent of the PON1-55 and 192 coding region polymorphisms which account for a large proportion of the activity variation in PON1 between individuals [9]. In a study of UK deployed veterans, serum PON1 activity was 25-35% lower than in non-deployed veterans which were not due to differences in PON1 coding region genotypes [10]. However, neither PON1 activity nor genotype was associated with specific symptoms of illness.

Due to the lack of association of PON1 activity or coding region polymorphisms with GWI, we have investigated whether promoter polymorphisms of the PON1 gene which have been reported to affect serum PON1 levels [11] are risk factors for GWI.

Materials and Methods

Subjects

The test population comprised 152 Gulf War Veterans recruited as described previously [9]. All had completed a questionnaire regarding 15 of the neurological and other symptoms previously associated with

GWI. The median number of symptoms present was 5 (3-13). The control population was 152 healthy individuals matched by gender and age within 5 years. None of the controls had served in the Gulf. The demographic details of the populations have been published previously [9]. The study was approved by the Central Manchester NHS Trust Research Ethics Committee.

Blood was obtained by venepuncture and serum, plasma and buffy coat prepared by low speed centrifugation as described previously [9].

Biochemical analysis

Serum PON1 activity was determined as hydrolysis of paraoxon in a continuously recording spectrometer at 405 nm as described previously [12]. Serum PON1 concentration was measured using our in-house competitive ELISA using rabbit antihuman PON1 monospecific antibodies [12]. DNA was extracted from the white cells of the buffy coat using the Puregene DNA isolation kit (Gentra systems, Milwaukee, USA) according to the manufacturers' instructions, the PON1- -108 and -909 promoter region polymorphisms were determined by PCR and restriction enzyme digestion using our standard published protocols [13].

Statistical analysis

Comparisons between groups were made by Student's unpaired t test. Non-gaussian parameters were logarithmically transformed before analysis. PON1 gene frequency was analysed by the Chi squared test.

Results

There were no differences in the distribution of the PON1 -108 or PON1 -909 genotypes between the Gulf War Veterans and the controls (Table 1), however, serum PON1 activity was 50% lower in the GWV compared to controls (100 (14.8- 233.8) vs 214.6 (50.3-516.2) nmol/min/ml (P<0.001) and PON1 concentration was also lower (75.7 (18.1-351.3) vs 88.2 (34.5- 527.4) µg/ml (P<0.00025).

GENOTYPE	CONTROLS	GWV
	Frequency (n)	Frequency (n)
-108 TT	0.25 (38)	0.26 (38)
TC	0.53 (79)	0.53 (82)
CC	0.22 (35)	0.21 (32)
-909 GG	0.16 (25)	0.21 (47)
GC	0.51 (77)	0.47 (74)
CC	0.33 (50)	0.32 (31)

Table 1: Genotype distribution in the Control and Gulf War Veteran (GWV) populations. No significant differences were found.

In the control population, PON1 activity was significantly different between the PON1 -108 genotypes in the order CC>TC>TT (P<0.001) and in the PON1 -909 genotypes in the order GG>GC>CC (P<0.001). However, in the Gulf War Veterans, such activity differences between genotypes were not evident (Table 2). In the Gulf War Veterans PON1 activity was significantly lower in all the PON1 -108 and -909 genotypes compared to controls (Table 2).

	CONTROL	GWV
PON1 Activity		
-108 TT	137.8 (50.3-516.2)	84.6 (27.8-243.5)+
TC	208.1 (73.3-487.2)	97.2 (14.8-222.2)+
CC	245.3 (78.6-620.8)*	112.2 (20.8-283.8)+
PON1 Mass		
-108 TT	87.3 (34.5-527.4)	61.4 (18.0-176.2)+
TC	87.9 (35.2-426.6)	76.7 (20.0-351.2)+
CC	94.5 (54.6-464.7)	78.4 (20.1-164.3)+
PON1 Activity		
-909 GG	302.8 (90.0-498.9)	103.4 (37.0-283.8)+
GC	212.2 (73.3-620.8)	104.5 (20.0-243.5)+
CC	217.7 (50.3-437.3)*	96.4 (14.8-221.9)+
PON1 Mass		
-909 GG	94.5 (52.0-464.7)	84.9 (19.3-164.3)•
GC	87.4 (35.2-462.4)	76.3 (18.1-351.2)•
CC	94.0 (34.5-527.4)	71.3 (26.0-232.1)•

Table 2: PON1 activity and mass according to genotype PON1 activity = nmol/min/ml PON1 mass = µg/ml

*Significance for trend P< 0.001

Significantly different from control + P< 0.001

• P< 0.05

There were no differences in PON1 concentration between any of the PON1 -108 or -909 genotypes in either population (Table 2), however, the concentration was lower in all genotypes in the Veterans compared to the controls.

Discussion

Serum PON1 levels are largely determined by polymorphisms in the coding and promoter regions of the gene, although a number of nutritional, pharmacological and lifestyle factors as well as diseases with an inflammatory component and/or insulin resistance can also modulate PON1 [14, 15].

This and previous studies have indicated that low serum PON1 activity and concentration are typical of populations symptomatic for GWI compared to matched controls. However, these differences in serum PON1 levels are not due to differences in the distribution of PON1 genetic polymorphisms in the coding region of the gene [9, 10]. Our current data suggests that differences in distribution of the promoter region polymorphisms are also not responsible for the low activity associated with GWI. Exposure to high concentrations of OPs has been shown to reduce serum PON1 [16], unfortunately, chemical exposures details were not available on our study group. However, our results do not allow us to rule out the possibility of gene-exposure interactions in the regulation of PON1 in GWI, as has recently been suggested to be the case with butyrylcholinesterase [17]. Nor can we

rule out the possibility of chemical exposures affecting microRNA regulation of PON1 [18] or epigenetic regulation of PON1 [19]. It has also recently been suggested that PON1 may be regulated in trans by an unknown gene found on chromosome 8 (p11, 21) [20]. These avenues require further investigation.

The question remains, therefore, as to whether PON1 is involved in GWI. Certainly in the case of atherosclerosis, where PON1 is believed to be antiatherosclerotic due to its ability to prevent the oxidation of lipoproteins and reduce the concentration of proatherogenic lipid-peroxides in the artery wall, as well as its ability to promote cholesterol efflux from vascular macrophages [21], low PON1 activity is associated with atherosclerosis development but genotypes associated with low PON1 activity are not.

Human toxicological studies have also produced conflicting results on the role of PON1 in OP toxicity [For detailed reviews see 1,22,23]. Added to this, a recent meta-analysis investigating the relationship between the PON1 coding region polymorphisms and OP toxicity, found that there was a significant association of the PON1-192QR polymorphism and OP toxicity in Caucasian populations but not in Oriental populations (which have drastically different Q and R genotype distributions compared to Caucasians) [24]. The frequency of the PON1-192R allele increases the further from Europe a population originates, increasing from 15-30% in Caucasians to 70-90% in Far Eastern Oriental and Sub-Saharan African populations [23]. Because the PON1-192R alloenzyme more efficiently hydrolyses many commonly used OPs compared to the PON1-192Q alloenzyme, Oriental populations will be better able to detoxify these OPs, theoretically leading to lower susceptibility to their toxic effects.

However, evidence is increasing for a link between OP exposure, low PON1 activity and neurological disturbances and chronic disease, particularly in children whose PON1 activity is approximately 4 times lower than in adults and which does not reach adult levels until after 7 years of age increasing children's susceptibility to OP toxicity [19]. It is therefore possible that OP exposure coupled with low PON1 activity could be responsible for certain GWV developing GWI, while others do not. Much larger epidemiological studies are warranted to investigate this possibility.

Conclusion

Low serum PON1 levels appear to be related to symptoms of GWI independently of PON1 promoter and coding region polymorphisms. More studies with larger cohorts are required to define a role for PON1 in GWI. The link between chemical exposure and low PON1 activity suggested by our studies on GWV also suggests the possibility of using PON1 as a diagnostic index of susceptibility to the development of neurological disturbances and chronic disease following chemical exposure of populations during major industrial accidents such as the recent Port of Taijin (China) disaster. Further studies in this area are clearly warranted.

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Conflict of Interest

There are none

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