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CASE REPORT

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Expression of paraoxonase isoform did not confer protection from acute sarin poisoning in the Tokyo subway terrorist attack

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Abstract We previously reported the polymorphism of the high density lipoprotein-associated enzyme paraoxonase (PON1), in the 10 sarin poisoning victims in the Tokyo subway terrorist attack. Arg_{192} PON1, which has low sarin hydrolysing activity, was found to be more common in the Japanese population than in people of other races. However, from our analyses seven of the victims expressed the PON1 phenotype with high sarin hydrolysing activity and three with low sarin hydrolysing activity. These results indicate that the main factor contributing to the tragedy of the Tokyo subway terrorist attack was the high toxicity of sarin rather than the race-dependent genetic difference in the Arg_{192} PON1 polymorphism.

Keywords Sarin · Paraoxonase · Sarin hydrolysing activity · PON1 · CAD

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Introduction

Paraoxonase (paraoxon hydrolase, PON1) is a high-density lipoprotein-associated enzyme capable of hydrolysing lipid peroxides and paraoxon. PON1 also hydrolyses nerve toxins such as soman and sarin and is known to be polymorphic in the human population. The PON1 enzyme activity polymorphism is substrate-dependent and varies among populations with different ethnic background [1]. The genetic basis of the inter-individual variability of PON1 activity has recently been attributed to the presence of an A-to-G polymorphism in the coding region of the gene (HUMPONA) coding for this enzyme. This polymorphism corresponds to a glutamine (Glu)/arginine (Arg) polymorphism at amino acid position 192 and the A and G variants code for glutamine (Gln_{192}) and arginine (Arg_{192}), respectively. The Arg_{192} PON1 isoform hydrolyses paraoxon rapidly, while the Gln_{192} isoform hydrolyses it slowly [1]. Conversely, the Gln_{192} isoform hydrolyses sarin more rapidly than the Arg_{192} isoform [2]. On the other hand, the PON1 genetic polymorphism has been suggested to be associated with the predisposition to coronary artery disease (CAD) [3].

A recent study reported that the Arg_{192} allele was more common in Japanese than in other races and described the possibility that this might have worsened the tragedy of the terrorist attack on the Tokyo subway with sarin [4, 5, 6].

To test this assumption, the following study was performed: forensic autopsies were carried out on the 10 sarin victims and the cause of death was diagnosed as acute sarin poisoning. In this paper, we demonstrate the allele frequency of the Arg_{192} allele in Japanese from Tokyo and the isoform distribution of PON1 in these 10 victims of sarin poisoning.

Materials and methods

The control subjects were 162 unrelated healthy adult Japanese individuals (94 male and 68 female) from the general population of

Table 1 PON1 genotypes of the victims and controls and the allele frequencies

Genotype	Victims (n = 10)	Control (n = 162)
Arg_{192}	3 (30%)	56 (34.6%)
Arg_{192}/Gln_{192}	6 (60%)	78 (48.1%)
Gln_{192}	1 (10%)	28 (17.3%)
Allele frequency		
Arg_{192}	0.60	0.59
Gln_{192}	0.40	0.41

Table 2 A profile of the 10 victims investigated

Case	Age (years)	Sex	Time of death after poisoning	PON1 type
1	29	Male	Instant death	Arg/Gln
2	51	Male	Instant death	Arg/Arg
3	51	Male	Approx. 20 h	Arg/Gln
4	64	Male	Approx. 2 days	Arg/Gln
5	52	Male	Approx. 15 months	Arg/Gln
6	42	Male	Instant death	Arg/Gln
7	50	Female	Instant death	Arg/Arg
8	95	Male	Instant death	Arg/Gln
9	33	Female	Instant death	Arg/Arg
10	54	Male	Approx. 10 h	Gln/Gln

Tokyo (Table 1) [7, 8, 9]. The volunteers had no symptoms of CAD or any other disease.

In order to prepare genomic DNA from the victims we took cardiac blood during the forensic autopsies of the 10 bodies. The survival times ranged from instant death to 15 months (Table 2). The techniques used for the extraction of DNA have been published previously [10, 11, 12].

PON1 genotyping

PON1 genotypes were determined using PCR and restriction mapping (162 unrelated healthy adult Japanese and the 10 victims); the nucleotide substitution corresponding to position 192 (Gln-Arg) creates an *Alw* I restriction site. A 99-base-pair fragment covering the region containing the mutation was amplified by PCR with the primers:

- 5′-TATTGTTGCTGTGGGACCTGAG-3′
- 5′-CACGCTAAACCCAAATACATCTC-3′

[1, 13]. PCR was performed by adding 0.5 µl of the extracted DNA to 45 µl of a mixture containing 1.25 pmol of each primer, 0.2 mM dNTP, 1 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% w/v gelatin) and 1 U Taq DNA polymerase (AmpliTaq Gold, Perkin-Elmer Cetus, Norwalk, Conn.). DNA was amplified for 35 cycles, each cycle comprising denaturation at 94 °C for 1 min, annealing at 61 °C for 30 s, extension at 72 °C for 1 min with a final extension time of 10 min (PCR System 2400, Perkin-Elmer). The PCR products were digested with 8 U of *Alw* I (Bio Labs, USA) restriction endonuclease according to the manufacturer's recommendation. The digested products were separated by electrophoresis on 3% agarose gel (Agarose HS, Wako Pure Chemical, Japan). DNA was visualised by SYBRGreen (FMC, USA) staining.

Results and discussion

Our preliminary experiments on the frequency of PON1 alleles in the general population of Tokyo showed that the Arg_{192} allele was very common, with an allele frequency of 0.59 (Table 1). The PON1 polymorphism has been recently implicated in another important area of human health. Serrato et al. have shown that the Arg_{192} allele represents a risk for CAD [6]. Therefore, we selected the volunteers who had no obvious symptoms of CAD [7, 8, 9]. We also compared this frequency with that in Hispanic (0.41) [2] and northern European (0.31) [14] populations. Approximately 35% of individuals from Tokyo were homozygous for the Arg_{192} PON1 isoform in contrast to 16% of Hispanic and 9% of northern European origin.

PON1 is the only human enzyme known to hydrolyse sarin. Humbert et al. [1] reported that a single amino acid mutation in acetylcholinesterase constituted the molecular basis of the activity polymorphism. In addition, they determined the phenotype by enzyme assay, which resulted in the enzyme activity of PON1 falling into three classes: low activity variant homozygous (Gln/Gln), heterozygous (Arg/Gln) and high activity homozygous (Arg/Arg). Conversely, Davies et al. [2] used the two-dimensional enzyme analysis and showed that the activity of PON1 with respect to the hydrolysis of diazoxon, soman and especially sarin was the opposite to that of paraoxon hydrolysis. The Arg/Arg form had low sarin hydrolysing activity and the Gln/Gln and the Arg/Gln forms had high sarin hydrolysing activity. The mechanism by which PON1 polymorphism influences resistance to sarin poisoning is speculative at present.

The detection of sarin hydrolysis products in the victims of the Tokyo subway sarin attack has already been reported [5, 6] and we have also made efforts to detect PON1 activity in the victims. However, the samples of blood were treated and frozen in several laboratories, so that it was not

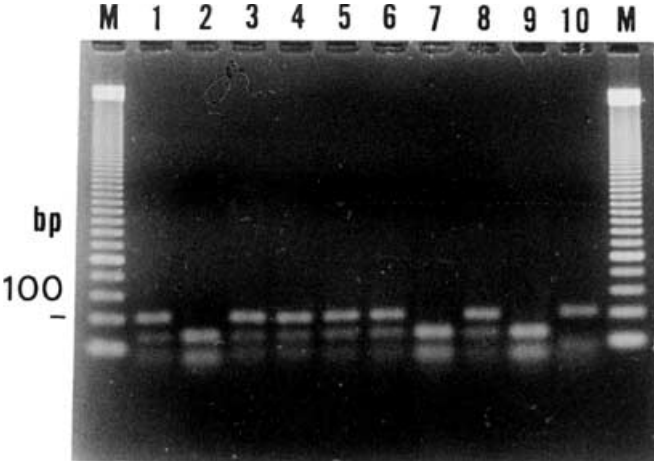

Fig. 1 Digestion of 99 bp PCR amplified fragments with restriction endonuclease *Alw* I. *Lanes M* 50-base-pair ladder, *lanes 1–10* 10 victims, *lanes 2, 7, 9* (Arg/Arg), *lanes 1, 3, 4, 5, 6, 8* (Arg/Gln), *lane 10* (Gln/Gln)

possible to determine PON1 activity using a neutral substrate such as phenyl acetate. Alternatively, we deduced the PON1 activity by identifying the genetic polymorphism. The prevalence of each allele for the Arg-Gln_{192} polymorphism among the 10 sarin victims was determined (Table 2). The results revealed an Arg_{192} allele frequency of 0.60 and the prevalence of the Arg_{192} allele was similar to that found in Tokyo populations (0.59, Table 1). Yamasaki et al. [4] described the possibility of a racial difference in vulnerability to sarin poisoning. However, they did not show any experimental data from the victims of sarin poisoning. According to our results, seven victims had the PON1 isoforms with high sarin hydrolysis activity PON1 (Gln/Gln and Arg/Gln) and three victims had low sarin hydrolysis activity (Arg/Arg) (Fig. 1, Table 2). We determined that death was due to the high toxicity of sarin rather than the race-dependent genetic control in the Arg_{192} PON1 polymorphism.

The PON1 polymorphism has been suggested to be associated with vascular disease and which also affects hydrolysis of the nerve agent sarin, but our results indicated that expression of the PON1 isoform with high sarin hydrolysis activity did not confer protection from acute sarin poisoning.

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