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Decreased paraoxonase-1 activity is a risk factor for ischemic stroke in Koreans

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Abstract

Paraoxonase-1 (PON1) is an enzyme associated with HDL in blood and it is considered as an anti-oxidant factor due to its capability to prevent lipid oxidation. *In vivo* mouse studies also have shown that PON1 is one of the genetic risk factors contributing to atherosclerosis. In this study, we evaluated the serum PON1 activities of sex–age matched Korean healthy control and ischemic stroke patients, and investigated the association of PON1 activity with other metabolic parameters. Statistical analyses revealed that PON1 activity and HDL_cholesterol (HDL_C) in stroke patients were significantly decreased when compared with those of healthy control. Additionally, PON1 activity was negatively correlated with age, whereas it was positively correlated with HDL_C in a stroke group. Overall, the results of this study indicated that decreased serum PON1 activity should be considered as a risk factor for ischemic stroke in Koreans.

Keywords: Stroke; Korean; PON1; Blood lipids

Human paraoxonase-1 (PON1) is an enzyme that is synthesized in the liver and then secreted into the blood [1]. PON1 has recently gained attention because it can hydrolyze and detoxify a variety of toxic cholinesterase inhibiting-organophosphorus (OP) compounds that are used for pesticides and nerve gases [2]. Three human *PON* genes (*PON1*, *PON2*, and *PON3*) have been identified and mapped in a cluster on chromosome 7q21.3 [3] and PON enzymes are associated with a high-density lipoprotein (HDL) surface [4]. One of PON family proteins, PON1, plays an important role in protecting HDL [5] and especially, low-density lipoprotein (LDL) from oxidative modification [4,6], which is known to be associated with many vascular diseases including atherosclerosis. In addition, previous data have shown that the inhibitory effect of PON1 on LDL oxidation may be anti-atherogenic because LDL oxidation is the major cause of atherogenic modification of serum LDL [7]. *In vivo* mouse studies revealed that *PON1* knockout (KO) mouse is more susceptible to atherosclerosis with high-fat and cholesterol diet as well as the toxicity of OP compounds when compared with wild type (WT) littermates [8], whereas transgenic (Tg) mouse overexpressing human PON1 has decreased atherosclerotic lesion formation [9]. Therefore, *PON1* is one of the genetic risk factors contributing to atherosclerosis and PON1 activity in blood should be considered as a blood parameter for diagnosis of atherosclerosis.

The identification of single nucleotide polymorphism (SNP) in the *PON1* gene including the promoter region is the most in-depth study elucidating the association of *PON1* polymorphism with coronary artery disease (CAD) [10] or cerebrovascular diseases (CVD) [11,12]. Several studies have reported that specific promoter or codon polymorphisms in the *PON1* gene are strongly associated with serum PON1 activities [7,13] or vascular diseases [14]. However, the association between serum PON1 activity and CVD is still obscure [15,16].

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CVD is responsible for more than 12.8% of the deaths in Korea, second to cancer (26.6%), and 70–80% of patients with CVD show stroke-related hemiplegia (data filed by the Korea National Statistical Office, 2005). Although data indicating the importance of PON1 activity on CVD within specific ethnic populations is growing, there is no study conducted on PON1 activity in the Korean population to date. Only one research group showed a relationship between *PON1* polymorphisms and serum lipid levels in Korean patients with CAD [17]. They showed that DdeI and AlwI polymorphisms within the *PON1* codon region were positively associated with HDL and LDL levels in men with CAD, respectively. However, they did not show any effects of SNPs on the actual PON1 concentration or enzyme activity in blood. Therefore, in this study, we investigated the PON1 activity and its association with other common metabolic parameters including serum lipids in healthy control and ischemic stroke patient group in Korean.

Materials and methods

Subjects and blood sample preparation. A total of 240 genetically unrelated, age (±2 years)–sex matched Korean subjects including 120 healthy control (65 males and 55 females; median age(interquartile range), 65(61,68)) and 120 ischemic stroke patients (66 males and 54 females; median age(interquartile range), 66(61,69)) were investigated in this study. Stroke patients with intracerebral (ICH) or subarachnoidal hemorrhage (SAH) were excluded, and ischemic stroke patients with large artery atherosclerosis (LAA), cardioembolism (CE) and small-vessel occlusion (SVO) were included in this study according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification. Written informed consent was received from all participants and all protocols were approved by the Institutional Review Board of the Korea Institute of Oriental Medicine. Fresh venous whole blood was collected from participants in Venous Blood Collection Tubes containing EDTA (BD Biosciences, Franklin Lakes, NJ, USA), and then separated into plasma and buffy coat containing white blood cells (WBC), platelets and red blood cells (RBC) by centrifugation at 1500*g* for 15 min. The cleared plasmas were immediately aliquoted and stored at −80 °C for further studies.

Chemicals. All commercially available chemicals used in this study were purchased from Sigma (St. Louis, MO, USA).

Serum PON1 activity. PON1 paraoxonase activity in the blood serum was determined by measuring the changes in spectrophotometric absorbance during enzyme–substrate reaction described by Beltowski et al. [18]. Briefly, substrate solution containing 0.1 M Tris–HCl, pH 8.0, 2.0 mM $CaCl_2$, and 2.0 mM paraoxon was freshly prepared immediately before the enzyme assay. Next, the aliquoted frozen plasmas were thawed slowly on ice, and then 20 μL of plasma was added to 800 μL of the substrate solution. The hydrolysis of paraoxon by PON1 activity was then monitored by observing the change in absorbance at 412 nm every 30 s for 10 min. PON1 activity is expressed in U/mL based on the extinction coefficient of *p*-nitrophenol, a product of paraoxon hydrolysis, at 412 nm (ε_{412}) of 18,290 $M^{-1}\,cm^{-1}$. One unit of PON1 can hydrolyze 1 nmole of paraoxon per min.

Serum metabolic parameters. Serum lipids such as total cholesterol (TC), HDL_cholesterol (HDL_C), and triglyceride (TG) were determined using automated biochemical analyzer (Spotchem™ SP-4410, Arkray Co., Kyoto, Japan). LDL_cholesterol (LDL_C) was calculated using the Friedewald formula, LDL ≈ TC − [HDL + TG/5]. Other blood parameters were measured using conventional biochemical methods.

Statistics. All statistical analyses were conducted using SAS software (version 9.1, SAS Institute Inc., NC, USA). All variables were tested for normality to determine analytical methods. Student's *t*-test and Wilcoxon rank-sum test were applied to normally distributed and not normally distributed variables, respectively, to test the differences between continuous variables. Chi-square statistic was used to test the difference between categorized variables such as sex. Spearman's rank correlation analysis was applied to determine the association of PON1 activity with clinical and serum metabolic parameters. A *p*-value of <0.05 was considered statistically significant.

Results and discussion

Study subjects

The clinical and common serum metabolic parameters of the healthy control and the ischemic stroke patient group evaluated in this study are summarized in Table 1. Some data, such as waist/hip ratio (WHR) for the stroke patient group, are not reported because physical examinations could not be conducted on severe ischemic stroke patients at the time of hospitalization. However, biochemical blood parameters were obtained from almost all patient subjects. The stroke patient group had a slightly greater WHR than the control group, however this difference was statistically significant ($p < 0.0001$), which indicates that the stroke patients are slightly obese. Among the serum lipids evaluated, only HDL_C was found to be significantly different between the control and stroke patients. In addition, a higher fasting blood sugar (FBS) was observed in the stroke patient group. This finding is consistent with the pathogenesis of ischemic stroke, which is a heterogeneous multifactorial disorder that is highly associated with metabolic disorders including diabetes, hyperlipidemia, obesity, hypertension and cardiovascular disease [19].

Table 1
Clinical and common metabolic parameters in healthy control and ischemic stroke patient group

Parameters	Control	Stroke	*p*
Sex (male/female)	65/55	66/54	ns
Age (years)	65(61,68) $N = 120$	66(61,69) $N = 120$	ns
WHR	0.88 ± 0.05 $N = 120$	0.94(0.90,0.98) $N = 60$	$p < 0.0001$
TC (mg/dL)	202.04 ± 39.30 $N = 120$	191.4 ± 42.2 $N = 117$	ns
TG (mg/dL)	143.5(112.0,195.5) $N = 120$	167.0(112.0,227.0) $N = 117$	ns
HDL_C (mg/dL)	47.5(39.2,55.1) $N = 120$	39.8(33.7,46.6) $N = 117$	$p < 0.0001$
LDL_C (mg/dL)	121.00 ± 35.94 $N = 120$	113.0 ± 36.4 $N = 117$	ns
FBS (mg/dL)	101.0(95,106.0) $N = 120$	124.0(103.0,168.0) $N = 117$	$p < 0.0001$

All results except sex are expressed as means±SD for parametric variables or as median(interquartile range) for non-parametric variables. Parametric Student's unpaired *t*-test or non-parametric Wilcoxon rank-sum test was used to compare the differences of variables between two groups. Chi-square test was used to compare the categorized variable (sex) between two groups. Abbreviations: *N*, number of observations; ns, not significant; WHR, waist/hip ratio; TC, total cholesterol; TG, triglyceride; HDL_C, HDL_cholesterol; LDL_C, LDL_cholesterol; FBS, fasting blood sugar.

PON1 activity in healthy and stroke patient groups

It has been reported that ischemic stroke is the most common form of stroke caused by atherosclerosis [20] and that atherosclerosis is initiated and propagated by oxidative modification of lipids [21]. Further, phospholipid oxidation of the LDL can lead to the initial fatty streak [22]. Currently, the role of PON1 *in vivo* is not well understood. However it has been suggested that PON1 can protect HDL and LDL from phospholipid oxidation [23,24]. Therefore, PON1 is considered as an anti-oxidant and anti-atherogenic factor.

Most previous reports have investigated the effects of two SNPs in the *PON1*coding region, Q192R and L55M [11,14–16,24], and two SNPs in the *PON1* promoter region, C(−107)T and G(−824)A [25,26], to determine if SNP mutations are associated with atherosclerosis or stroke based on the assumption that mutation in the coding and promoter regions of *PON1* can alter the transcription efficiency, protein stability, or enzyme activity, thereby influencing the development of atherosclerosis and stroke. However, the results of these studies have been the subject of debate, possibly due to the ethnic specificity and properties of the study subjects, such as age, life styles, family history, and disease models used. Therefore, the actual PON1 concentration or enzyme activity, rather than SNP mutation of *PON1* gene, may be a more useful indicator of atherosclerosis or stroke.

The serum paraoxonase activity of PON1 in the control and stroke patient group are shown in Fig. 1 and summarized in Table 2. The overall enzyme activity of the healthy Korean population was lower than that of the European population [27] and comparable to that of other Asian populations [28]. The PON1 paraoxonase activity of the stroke patient group (median(interquartile range),

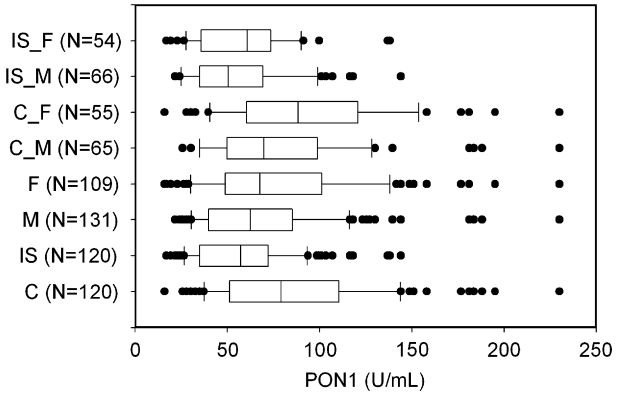

Fig. 1. PON1 paraoxonase activities against the synthetic substrate, paraoxon, in healthy control, and ischemic stroke patient group. The medians (vertical solid lines), 25–75th percentiles (boxes), and 95th percentiles (whiskers) of PON1 paraoxonase activities are shown in box plots. Abbreviations: *N*, number of observations; C, healthy control; IS, ischemic stroke patient group; M, pooled male; F, pooled female; C_M, male within a healthy control; C_F, female within a healthy control; IS_M, male within an ischemic stroke patient group; IS_F, female within an ischemic stroke patient group.

Table 2
Serum PON1 paraoxonase activity in healthy control and ischemic stroke patient group

	Group			Sex			Group					
							Control			Stroke		
	Control ($N = 120$)	Stroke ($N = 120$)	p^*	Male ($N = 131$)	Female ($N = 109$)	p	Male ($N = 65$)	Female ($N = 55$)	p	Male ($N = 66$)	Female ($N = 54$)	p
Paraoxonase (U/mL)	79(51, 110)	57(35, 72)	<0.0001	62(40, 85)	67(49, 100)	ns	70(51, 41)	88(60, 47)	ns	50(35, 68)	61(36, 73)	ns

All results are expressed as mean±SD for parametric variables or as median (interquartile range) for non-parametric variables.
* *p*-values by non-parametric Wilcoxon rank-sum test. Abbreviations: *N*, number of observations; ns, not significant.

57(35, 72)) was much lower than that of the healthy control group (median(interquartile range), 79(51, 110)) with the statistical significance ($p < 0.0001$). These results are consistent with those of a recent study showing that acute ischemic stroke patients with a QRLL or RRLL genotype of Q192R and L55M had lower PON1 activity than control subjects with the same genotype [29]. However, the PON1 activity profile was not affected by sex within the pooled or grouped (control or stroke) populations (Table 2). Another PON1 activity, arylesterase, determined by using different synthetic PON1 substrate, phenylacetate, was lower in the stroke patient group than in the control group. However, this difference was not statistically significant (data not shown). These discordant results of PON1 activity against two synthetic substrates, paraoxon and phenylacetate, were also consistent with the results of a previous study conducted on the association of *PON1* SNP mutation with PON1 activity and diabetes complications [13]. These results may be due to the two enzyme activities of PON1, paraoxonase and arylesterase, being controlled by different regulating mechanisms or having different substrate accessibility (see more information in the review paper by Beltowski [30]). In addition, Sumegova et al. demonstrated that PON1 arylesterase activity is marginally affected by sex in the healthy Slovak population [31].

Correlation of serum PON1 activity level with clinical and serum metabolic parameters

Spearman's rank correlation analysis was conducted to investigate the association of PON1 activity with other clinical and serum metabolic parameters. The correlation coefficients (r) and p-values are summarized in Table 3. In the pooled population, irrespective of stroke disease status or sex, PON1 activity was negatively correlated with age ($r = -0.1620$, $p = 0.0120$), WHR ($r = -0.2909$, $p < 0.0001$) and FBS ($r = -0.2135$, $p = 0.0010$). The decreased PON1 activity in the population with a high FBS is consistent with the results of a previous study evaluating PON1 activity in patients with diabetes mellitus [13]. PON1 activity was positively correlated with TC ($r = 0.2467$, $p < 0.0001$), HDL_C ($r = 0.2546$, $p < 0.0001$), and LDL_C ($r = 0.2177$, $p = 0.0007$). Interestingly, the correlation of PON1 activity with serum metabolic parameters in the pooled population was also observed in mammals other than humans in the same manner [32]. The correlation of PON1 activity with clinical and metabolic parameters differed according to the stroke disease status or sex. For example, the negative correlation of PON1 with age shown in the stroke patient group was not observed in the control group ($p = 0.5356$), which is in agreement with other published data regarding Asian populations [29]. Further, PON1 activity was correlated with WHR ($r = -0.2152$, $p = 0.0183$), TC ($r = 0.2450$, $p = 0.007$), and LDL_C ($r = 0.2099$, $p = 0.0214$) in the control group, whereas it was correlated with age ($r = -0.2511$, $p = 0.0057$) and HDL_C ($r = 0.2029$, $p = 0.0283$) in the stroke patient group. The correlation observed in the pooled population, irrespective of stroke disease status or sex, was also observed in the female group in the same manner. However, only WHR was found to be correlated with PON1 ($p = 0.0247$) in the male group. Although the

Table 3
Correlation of PON1 paraoxonase activity with clinical and serum metabolic parameters

		Total	Group		Sex		Group			
							Control		Stroke	
			Control	Stroke	Male	Female	Male	Female	Male	Female
Age	r*	−0.1620	−0.0571	−0.2511	−0.1344	−0.2120	−0.0145	−0.1234	−0.2085	−0.3120
	p	**0.0120**	0.5356	**0.0057**	0.1258	**0.0269**	0.9085	0.3695	0.0930	**0.0216**
	N	240	120	120	131	109	65	55	66	54
WHR	r	−0.2909	−0.2152	−0.1153	−0.2315	−0.3223	−0.1819	−0.0885	−2963	−0.0070
	p	**<0.0001**	**0.0183**	0.3803	**0.0247**	**0.0025**	0.1470	0.5204	0.1186	0.9703
	N	180	120	60	94	86	65	55	29	31
TC	r	0.2467	0.2450	0.1700	0.1499	0.2946	0.2153	0.1924	−0.0136	0.3077
	p	**0.0001**	**0.0070**	0.0670	0.0888	**0.0021**	0.0850	0.1593	0.9144	**0.0265**
	N	237	120	117	130	107	65	55	65	52
TG	r	0.0273	−0.0044	0.1229	0.0476	0.0263	−0.0659	0.1640	0.1310	0.1232
	p	0.6754	0.9617	0.1868	0.5911	0.7880	0.6018	0.2316	0.2983	0.3843
	N	237	120	117	130	107	65	55	65	52
HDL_C	r	0.2546	0.1244	0.2029	0.1535	0.2861	0.1489	−0.0476	−0.0082	0.4327
	p	**<0.0001**	0.1758	**0.0283**	0.0813	**0.0028**	0.2364	0.7301	0.9481	**0.0014**
	N	237	120	117	130	107	65	55	65	52
LDL_C	r	0.2177	0.2099	0.1365	0.1518	0.2313	0.2135	0.1272	0.0012	0.2151
	p	**0.0007**	**0.0214**	0.1424	0.0847	**0.0165**	0.0878	0.3547	0.9922	0.1258
	N	237	120	117	130	107	65	55	65	52
FBS	r	−0.2135	−0.0999	−0.0243	−0.0635	−0.3799	0.0884	−0.2675	0.0248	−0.0618
	p	**0.0010**	0.2777	0.7964	0.4764	**<0.0001**	0.4837	**0.0484**	0.8469	0.6633
	N	235	120	115	128	107	65	55	63	52

* r, Spearman's rank correlation coefficient and p-values with statistical significance (<0.05) are shown in bold.

different patterns of PON1 correlation with other parameters between male and female groups may be due to sex hormonal effects and metabolic changes, further studies should be conducted to evaluate this difference in patterns.

In conclusion the serum PON1 activity in ischemic stroke patients is much lower than that of the healthy population and therefore, PON1 activity should be considered as a risk factor for ischemic stroke in Koreans. Identification of the mechanism controlling PON1 activity will provide opportunities for the development of new therapeutics or preventives for ischemic stroke.

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