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In vitro Effects of Salicylic Acid on Plasma Paraoxonase 1 Activity

Dhouha Haj Mouhamed[1,2]*, Asma Ezzaher[1,2], Lotfi Gah [2], Wahiba Douki[1,2] and Mohamed Fadhel Najjar[1]

[1]*Laboratory of Biochemistry-Toxicology, Monastir University Hospital, Monastir 5000, Tunisia*
[2]*Research Laboratory "Vulnerability to Psychotic disorders LR 05 ES 10", Department of Psychiatry, Monastir University Hospital, Tunisia*

Abstract

Objectives: Our study aimed to examine the effects of salicylic acid on paraoxonase activity in healthy volunteers. **Methods:** Plasma from healthy volunteers was spiked with salicylic acid drug. The working solutions were then diluted with plasma to obtain concentrations that covered the therapeutic margin. Specimens were incubated at 25 and 37°C, and we measured PON1 activities in the presence of different drug concentrations after 1 h, 3 h, and 24 h and after one week.

Results: After incubation *in vitro,* a significant increase of paraoxonase 1 activity according to salicylic acid concentration and incubation duration, regardless of temperature incubation was showed. This increase was marked especially after 24 h of incubation. The stimulatory effect of salicylic acid is concentration and incubation duration-dependent and does not seem to be related to temperature incubation.

Conclusion: The tested Salicylic acid significantly stimulated PON1 activity in a concentration dependent manner. This result shows that acetyl salicylic acid treatments have a beneficial effect dose and time dependent that may contribute to prevent atherosclerosis.

Keywords: PON1 activity; *In vitro*; Salicylic acid

Introduction

Salicylic acid (SA) is used to prevent complications of atherosclerotic cardiovascular disease such as myocardial infarction and occlusive stroke [1]. Salicylic acid treatment has been reported to result in a 32% reduction in the risk of a first myocardial infarction. Also, this drug is used in the treatment of patients with acute coronary syndromes. The above beneficial effects of acetyl salicylic acid (ASA) are generally attributed to its platelet-inhibitory function [1]. However, other platelet inhibitory agents have not been found to be effective or as effective as aspirin. Therefore, the discrepancy between the efficacy of these compounds and aspirin suggests that the therapeutic efficacy of aspirin may not be limited only to its platelet inhibitory effect [2]. Human serum paraoxonase catalyses the hydrolysis of organophosphates, aromatic carboxylic acid esters, lactones, and carbamates, in a calcium-dependent manner. Paraoxonase 1 is synthesized in the liver and is thought to be exclusively associated with high-density lipoprotein in the plasma. Paraoxonase 1, a high-density lipoprotein associated antioxidant enzyme, has been shown to protect the serum lipids from oxidation and can reduce macrophage foam cell formation and attenuates atherosclerosis development. Human serum PON1 activity was reported to be inversely related to the risk of coronary artery disease (CAD) [3]. Its activity decreases in cardiovascular disease and in atherosclerotic, hypercholesterolemic, hypertensive and diabetic patients [3]. Our study aimed to investigate the *in vitro* effects of salicylic acid on human plasma paraoxonase 1 activity.

Materials and Methods

Plasma samples

Blood samples were taken from healthy volunteers who were not being treated with any drugs and were drawn in tubes containing lithium heparinate. Plasma was obtained after centrifugation of the whole blood for 15 min at 3000 rpm.

Solutions

Stock solutions (2g/L) of salicylic acid drug were prepared in water and stored at -20°C. All stock solutions were further diluted with water to produce working solutions. These working solutions were diluted with plasma to obtain the desired final concentrations that covered the therapeutic margin of salicylic acid (2000, 1600, 800, 400 and 100 mg/L). The plasma control (control activity) was prepared by adding water to plasma (v/v).

In vitro drug studies

We examined the stimulatory effects of salicylic acid drugs. The pool of plasma was spiked with the solution of salicylic acid as previously described. All compounds were tested in triplicate at each concentration used. Specimens were incubated at 25 and 37°C, and PON1 activities were measured in the presence of different drug concentrations after 1 h, 3 h, and 24 h and after one week. Control activity was considered to be 100% in the absence of SA.

Assay of paraoxonase 1 activity

PON1 activity was measured using paraoxon as substrate by adding plasma to 0.5 mL of 50 mM Tris buffer (pH 7.4) containing 1 mM $CaCl_2$ and 1 mM paraoxon. One unit of PON 1 activity is defined as 1 µ mol of product generated per min. The enzyme assay was based on the estimation of p-nitro phenol at 412 nm at 25°C. Assays were performed on a Konelab 30™ (Thermo Electron Corporation, Ruukintie, Finland) [4].

Statistical analysis

We use the Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA). All data are expressed as mean ±

***Corresponding author:** Dhouha Haj Mouhamed, Laboratory of Biochemistry-Toxicology, University Hospital of Monastir 5000, Tunisia, Tel: (216) 73106000; Fax: (216) 73460678; E-mail: hajdhouha@yahoo.fr

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standard deviation (SD). The significance of differences in PON1 activity (drug-treated samples *vs.* control) was estimated using Student's t-test ($p<0.05$). A stimulation percentage of PON1 activity > 10% was considered as significant.

Results

PON1 activity in the control plasma was stable over the experiment duration. We did not find significant differences between PON1 activity values measured after 1 h, 3 h, and 24 h and after one week in the control tube.

After *in vitro* incubation, we showed a significant increase of paraoxonase 1 activity according to salicylic acid concentration and incubation duration, regardless of temperature incubation. This increase was marked especially after 24 h of incubation (Tables 1 and 2).

The stimulatory effect of salicylic acid is concentration and incubation duration-dependent and does not seem to be related to temperature incubation. After an incubation time of on week, the stimulation percentage for salicylic acid was 43% at the low concentration of 50 mg/L (400 ± 1 IU/L *vs.* 259 ± 2 IU/L; $p<0.001$) and 50% at 1 g/L (414 ± 1 IU/L *vs.* 259 ± 2 IU/L; $p<0.01$) (Figures 1 and 2).

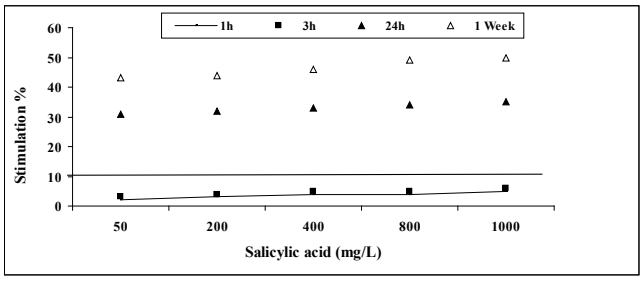

Figure 1: Stimulation percentage of PON1 activity according to concentration of salicylic acid drugs and incubation duration at 25°C.

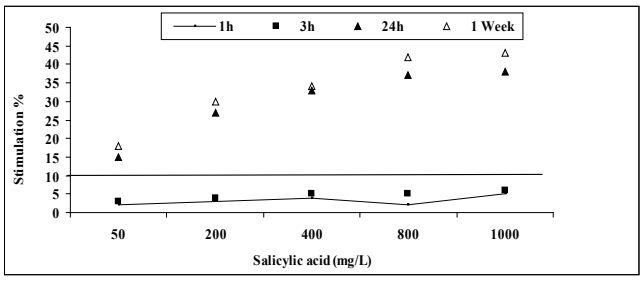

Figure 2: Stimulation percentage of PON1 activity according to concentration of salicylic acid drugs and incubation duration at 37°C.

Incubation duration		*Controls*	*salicylic acid concentration (mg/L)*				
			50	**200**	**400**	**800**	**1000**
1h	M ± SD	259 ± 1	262 ± 8	271 ± 1	267 ± 7	262 ± 1	276 ± 8
	p	-	0.4	0.009	0.1	0.3	0.03
3h	M ± SD	259 ± 1	266 ± 6	269 ± 4	262 ± 1	261 ± 1	269 ± 7
	p	-	0.08	0.02	0.07	0.06	0.02
24h	M ± SD	260 ± 1	369 ± 2	370 ± 1	372 ± 1	375 ± 2	376 ± 6
	p	-	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$
1 week	M ± SD	259 ± 1	400 ± 1	402 ± 1	407 ± 1	414 ± 1	418 ± 3
	p	-	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$

Table 1: PON1 activity (IU/L) according to salicylic acid concentration and time of incubation at 25°C.

Incubation duration		*Controls*	*salicylic acid concentration (mg/L)*				
			50	**200**	**400**	**800**	**1000**
1h	M ± SD	259 ± 1	264 ± 1	267 ± 1	269 ± 4	264 ± 1	271 ± 4
	p	-	0,4	0.009	0.02	0.3	0.01
3h	M ± SD	259 ± 1	270 ± 1	270 ± 1	271 ± 1	272 ± 6	273 ± 1
	p	-	0.01	0.01	0.01	0.02	$< 10^{-3}$
24h	M ± SD	260 ± 1	326 ± 1	358 ± 1	372 ± 1	384 ± 1	386 ± 3
	p	-	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$
1 Week	M ± SD	259 ± 1	335 ± 9	364 ± 8	376 ± 6	395 ± 4	398 ± 7
	p	-	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$

Table 2: PON1 activity (IU/L) according to salicylic acid concentration and incubation duration at 37°C.

Discussion

Toxicology, enzyme-drug interactions and chemical interaction studies with various enzymes are vital, and the number of these studies is increasing every day worldwide. In general, drugs and toxic substances exhibit their biological effects through interaction with specific enzymes. In the present study, we investigate the *in vitro* effects of salicylic acid drug on PON1 activity.

We noted that PON1 activity in the control plasma was stable over the experiment duration, and there were no significant differences in the PON1 activity measured after 1 h, 3 h, 24 h and after one week.

After *in vitro* incubation, we showed a significant increase of paraoxonase 1 activity according to salicylic acid concentration and incubation duration, regardless of temperature incubation. This increase was marked especially after 24h and one week of incubation. These results confirm those of Kurban and Mehmetoglu [3], which showed that the activity of PON was higher in volunteers treated with salicylates for one week. Recent studies [5] reported that aspirin users have increased PON activity in the plasma and results of Cyrus et al. [6] and Maree and Fitzgerald [7] suggest that aspirin is an antiatherogenic drug. The principal mechanism explaining the beneficial effect of aspirin in preventing cardiovascular disease is its ability to prevent the formation of thromboxanes and platelet aggregation. In addition, aspirin has been suggested to exert to acetylating the key enzymes. Accordingly, cyclooxygenases have been reported to be inhibited by aspirin [8]. But, aspirin's effects on platelets alone cannot explain its ability to inhibit atherosclerosis. Indeed, there is very little evidence to indicate that involves aggregated platelets until the terminal consequences of plaque rupture [3].

Paraoxonase 1 is an HDL-associated esterase/lactonase, and its activity is inversely related to the risk of cardiovascular disease. ApoA1, the major protein in HDL, stabilizes PON1, and binds it with a very high affinity [9,10]. In recent years, interactions between drugs and human paraoxonase interested many researchers that have tested different xenobiotics effects *in vitro* on PON1 [11]. Antibiotics including ceftriaxone, ceftazidime and sulfonamides and furosemide, were confirmed to decrease the *in vitro* enzyme activity at different concentrations [12,13]. Intravenous anesthetics, etomidate, propofol and ketamine were evidenced to dose-dependently decrease *in vitro* PON1 activity [14]. The cholinergic muscarinic antagonist atropine was also shown to inhibit PON1 *in vitro* at high concentrations [15]. PON1 activity inhibitions by cigarette smoke extract in vitro and in vivo human are dose- and time-dependent manner [10,16]. Several metal

ions, such as cobalt, cupper, iron, lead, manganese and mercurials, have been shown to decrease *in vitro* PON1 activity [12,13,17]. The list of tested compounds continues to grow longer. Researchers interested in the effects of antidepressants on PON1 activity have only performed in vivo studies and they emphasized the potential antioxidant activity of selective serotonin re-reuptake inhibitors, such as lithium, in bipolar disorder [18].

In this study, we noted that the stimulatory effect of salicylic acid is concentration and incubation duration-dependent and does not seem to be related to temperature incubation. The stimulation percentage for salicylic acid was 43% at the low concentration of 50 mg/L after an incubation time of on week. At 1 g/L, the stimulation percentage for salicylic acid was 50% after one week of incubation. This results are similar than those found by Costa et al. [15]. They have explained this increase by the effect of nutritional, pharmacological, environmental factors and drugs on serum PON1 activity and PON1 gene. The induction of PON1 by salicylate provides elucidation of the additional mechanisms that allow to aspirin to protect against atherosclerosis. This stimulating effect of paraoxonase, partly explain the cardioprotective actions of salicylic acid. Indeed, salicylate therapy has become essential, in combination with other treatment modalities, for those with established risk factors for cardiovascular diseases. Salicylate is an analgesic, antiinflammatory, and antipyretic drug, and also inhibits the cyclooxygenase enzyme by acetylation of its active site. The pharmacological effects of salicylate are due to the inhibition of the formation of cyclooxygenase products, including prostaglandins, thromboxanes, and prostacyclin. The beneficial effects of salicylate on atherosclerosis cannot be solely attributed to its platelet-inhibitory function, because the aggregation of platelets contributes little to experimental atherosclerosis [19].

Conclusions

The tested salicylic acid significantly stimulated PON1 activity in a concentration dependent manner. This result shows that acetyl salicylic acid treatment have a beneficial effect dose and time dependent that may contribute to prevent atherosclerosis.

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