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STATE-OF-THE-ART

Paraoxonases as Protective Agents Against N-Acyl Homoserine Lactone – Producing Pathogenic Microorganisms

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ABSTRACT

Paraoxonases are a group of enzymes with a high „substrate-promiscuity", being able to act on many structurally different compounds. To date, there is no consensus regarding the physiological substrate(s) of these enzymes. Recent data suggest that the N-*acyl homoserine lactones (AHLs) produced by different Gram-negative bacteria, including the opportunistic* Pseudomonas aeruginosa, *could be such substrates. Due to the ability of paraoxonases to hydrolyze AHLs, they represent an alternative mechanism of protection against pathogen microorganisms, interfering with the quorum sensing systems that allow these bacteria to respond in a coordinate manner to different changes in the extracellular environment. This mini-review presents some novel aspects regarding the relationship between paraoxonases and the aforementioned compounds, highlighting the potential role of these enzymes as a component of the humoral innate defence system.*

Keywords: paraoxonases, acylhomoserine lactone (AHLs), quorum sensing, sepsis

INTRODUCTION

During the past several years, researchers focused on revealing the role of paraoxonases in the protection of human body against AHLs-producing pathogenic microorganisms. Different in vitro and in vivo studies indicated that these enzymes are able to protect against infections, offering thus a potentially treatment alternative. These studies offered also a possible clue to the paraoxonases blood status in critically ill patients.

Paraoxonases – enzymes in search for a substrate

Paraoxonases are a group of enzymes (PON1, PON2, and PON3) highly conserved in vertebrates and in particular in mammals (1). In humans, the corresponding genes (*PON1*, *PON2* and *PON3*, respectively) are located on the same region of the chromosome 7 (7q21-22) (2). It is believed that these genes evolved through a process of duplication. From a phylogenetic point of view, *PON2* was the first gene that appeared, followed by *PON3*, and *PON1*

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as the most recent (3). In humans, *PON1* and *PON3* are mainly expressed in liver and kidney cells, while *PON2* is expressed in a broad range of tissues (3). PON1 and PON3 are high-density lipoprotein particles (HDL)-associated proteins, while PON2 has an intracellular localization, in cells of the brain, kidneys, and testis, among other tissues (4-6). A recent study indicated that PON1 can be transferred from the HDL particles to the external face of the cellular plasma membrane without losing the enzymatic activity (7).

Paraoxonases are characterized by a high „substrate-promiscuity", i.e. they act on many different substrates (3,8). The term „paraoxonase" was coined after the discovery of a serum enzyme able to hydrolyze paraoxon, a metabolite of parathion (9,10). This very first enzyme was termed PON1, and the other two which were identified in biological samples became PON2 and PON3. Despite its extensive use, this term does not reflect the physiological function of these enzymes. Further studies revealed that PON1 was able to hydrolyze other organophosphates (chlorpyriphos, diazoxon) and even nerve agents (sarin, soman) (11,12). PON1 has also the ability to hydrolyze aromatic esters (phenylacetate, thiophenylacetate, 2-naphtylacetate) (12). However, the other two members of the paraoxonase family have a very limited activity towards paraoxon and aromatic esters. On the other hand, it was found that all paraoxonases are actually lactonases, being able to act on a variety of aliphatic and aromatic lactones (homogentisic acid lactone, dihydrocoumarin, γ-butyrolactone, homocysteine thiolactone, 5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid, and 4-hydroxy-5E,7Z,10Z,13Z,16Z,19Z-docosahexanoic acid) (13-16). Moreover, it was found that PON1 is able to catalyze the formation of lactones (a lactonization reaction) from γ- and δ-hydroxyacids (Figure 1) (16,17).

Recent studies indicated that paraoxonases are able to hydrolyze compounds belonging to the class of AHLs, which are synthesized and secreted in the extracellular space by some Gram-negative bacteria (7,18-20). AHLs are involved in the phenomenon of quorum-sensing (21).

The concept of quorum sensing

Despite the long-lived belief that there is no communication between the members of a population of microorganisms, today this phenomenon, called quorum-sensing is well documented. Different microorganisms are able to produce, release in the extracellular space and detect signal molecules in response to the alteration of the population's density. These signals allow unicellular organisms to function as multicellular organisms.

From a structural point of view, these signals are very diverse, ranging from N-acyl homoserine lactones (AHLs), hydroxylated and unsatu-

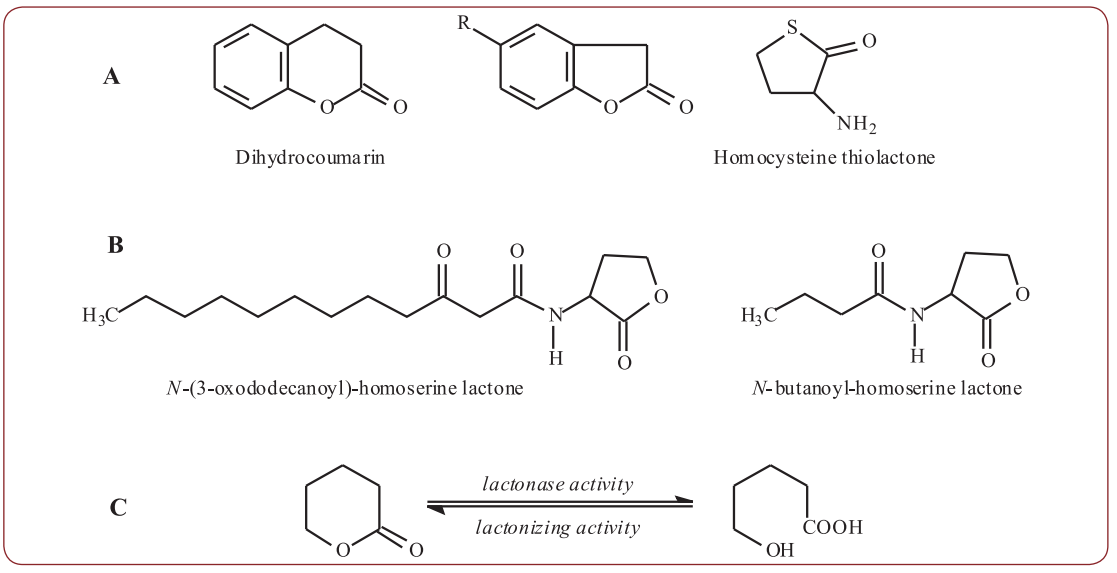

FIGURE 1. B(A) Lactones used as substrates by paraoxonases (R=H, 2-coumaronone; R=OH, homogentisic acid lactone). (B) The *N*-acyl homoserine lactones produced by *P. aeruginosa*. (C) Lactonase and lactonazing activities exemplified on the 5-hydroxy-pentanoic acid. (modified from reference 13).

rated fatty acids, to cyclic and linear peptides (22). These molecules alter the expression of different genes involved in processes like competence induction, bioluminescence, secretion of virulence factors, biofilm formation and sporulation (21).

The quorum sensing system that uses AHLs is very well characterized, being described in many Gram-negative bacteria of which some have agricultural importance (*Agrobacterium tumefaciens, Erwinia carotovora*), and other have medical importance (*Pseudomonas aeruginosa, Burkholderia sp.*) (22). The signal molecules used by this system are N-acylated derivates of homoserine lactone with fatty acids (C_4-C_{18}). Acylation with fatty acids with less than C_4 lead to the loss of the biological function of an AHLs (23).

P. aeruginosa, an opportunistic bacterium responsible for nosocomial infections, can also affect immunosuppressed and HIV/AIDS patients, as well as cancer patients undergoing chemotherapy (24). Patients with burns are another group with high risk of infection with P. aeruginosa (25). *P. aeruginosa* can also disseminate systemically leading to sepsis. Almost 15% of the diabetes patients develop foot ulcers which are very susceptible to infections, *P. aeruginosa* infections being encountered in approximately 3.5% of cases (26). Moreover, *P. aeruginosa* is responsible for catheter-related infections (27).

P. aeruginosa has two well described quorum sensing systems, both requireing an autoinducer molecule that acts on a specific protein involved in regulation of gene expression (28). The las system has as an autoinducer *N*-(3-oxododecanoyl) homoserine lactone (3OC12-HSL) that acts on LasR, while the *rhl* system uses *N*-butanoyl homoserine lactone that acts on RhlR (Figure 1). Both LasR and RhlR proteins control the expression of genes coding for different virulence factors (exotoxin A, alkaline protease, elastase, pyocyanin, type I and II lectins, chains A and B of rhamnosyltransferase). Moreover, the *rhl* system is transcriptionally and post-translationally controled by the *las* system.

Studies linking the paraoxonases and infections

Recent studies indicated that different AHLs can be degraded by serum samples from mammalian species (human, rabbit, mouse, horse, goat, and bovine) (29). *In vitro* and *in vivo* studies with human epithelial cells, human hepatoma cells (HelG2), murine and *Drosophila melanogaster* models indicated that paraoxonases are able to inactivate a broad range of AHLs. The great majority of these studies focused on the quorum sensing systems used by *P. aeruginosa*. It was found that all three human paraoxonases are able to hydrolyze 3OC12-HSL in the following order of efficiency: PON2 >> PON1 > PON3 (20). Deletion of *PON2* was followed by the impairment of the 3OC12-HSL inactivation by murine tracheal epithelial cells lysates when comparing to wild-type mice (18). Moreover, an *in vitro* study indicated that murine PON1 is able to inhibit the formation of *P. aeruginosa* biofilms (30).

Another approach to study the relation between pathogenic microbes that produce AHLs, as part of an quorum sensing system, and human paraoxonases is the fruit fly (*Drosophila melanogaster*) (1,19). The advantage is that *D. melanogaster* lacks paraoxonases and does not express paraoxonases homologues (1). Transgenic flies expressing human PON1 exhibited lactonase activity and were able to hydrolyze 3OC12-HSL, being thus protected from *P. aeruginosa* infection (19). Also, PON1 protected the transgenic flies against the infection with *Serratia marcescens,* which also produces AHLs (1).

Paraoxonases are able to hydrolyze the AHLs that are produced by many other pathogenic microorganisms belonging to different genera, i.e. *Aeromonas sp., Brucella sp., Burkholderia sp., Serratia sp., Yersinia sp.* (31).

A recent study indicated that in critically ill patients both paraoxonase and arylesterase activities were significantly decreased when compared to healthy controls (32). ❑

CONCLUDING REMARKS

These data suggest that paraoxonases can offer an alternative mechanism to the innate and acquired immunity through interfering with the quorum sensing system of AHLs-producing pathogenic microorganisms. Thus, these enzymes can become an alternative tool against some infectious diseases.

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