Are prostatitis symptoms associated with an isoprostane-mediated vicious circle?

Silver Türk a,⇑, Tiiu Kullisaar b

a Department of Microbiology, Tartu University, Ravila 19, Tartu 50411, Estonia
b Department of Biochemistry, Tartu University, Ravila 19, Tartu 50411, Estonia

Introduction

The main symptom of prostatitis is pain, which is often accompanied by lower urinary tract symptoms and sexual disturbances. In nine cases out of 10, the etiology of prostatitis is unclear, and such patients are diagnosed with chronic prostatitis. Prostatitis has been explained by infectious, autoimmune and neuromuscular etiologies [1]. Recently, OxS (oxidative stress) has been detected in prostatitis patients. This paper aims to identify the precise putative pathways that link OxS with prostatitis symptoms.

As concerns treatment of prostatitis, the therapy usually consists of fluoroquinolones, α-adrenoblockers and possibly over-the-counter painkillers. Importantly, the widely used fluoroquinolone treatment has not provided benefit for the patients [2,3] and the benefit of conventional anti-inflammatory agents has been controversial [4–6]. Benefit has been provided by antioxidants [7–9]. That argues in favor of a higher pathogenetic relevance of OxS.

Systemic OxS has been observed in all four subtypes of prostatitis: chronic bacterial prostatitis [10], inflammatory CPPS (chronic pelvic pain syndrome) [11], non-inflammatory CPPS (unpublished data) and asymptomatic inflammatory prostatitis [11].

Isoprostanes are stereoisomers of prostaglandins that form primarily by non-enzymatic peroxidation of arachidonic acid by reactive oxygen species (ROS) [12]. Isoprostanes differ in their half-life, stability, chemical properties and affinity towards prostaglandin receptors. That causes different profiles of bioactivity for isoprostanes. 8-Isoprostanes are relatively stable and excrete with urine, making them excellent if not the best markers of systemic OxS. However, 8-isoprostanes are a tip of an iceberg. It has been shown that the level of 8-isoprostanes in brain is sevenfold exceeded by the level of electrophilic cyclopentenone isoprostanes, even more in the case of marked injury or OxS, even though such electrophiles are chemically less stable [13]. Of LPP (lipid peroxidation products), isoprostanes are relevant for pain because they can upregulate nociceptive pathways by stimulating prostaglandin receptors [12]. The pertinent pathways that innervate prostate, bladder and urethra express nociceptors TRPA1 (transient receptor potential cation channel, subfamily A, member 1) and TRPV1 (transient receptor potential cation channel, subfamily V, member 1) [14–16]. Cyclopentenone isoprostanes and other electrophilic LPP are relevant because they activate nociceptor TRPA1 [17,18] and react with intracellular thiols [13] including glutathione, which is the primary nonenzymatic intracellular antioxidant.

⇑ Corresponding author. Tel.: +372 7374185; fax: +372 7374172.
E-mail address: silver.turk@mail.ee (S. Türk).
Previously, it has been shown that a peripheral injury causes sensitisation that is associated with mitochondria-generated OxS in the dorsal horn of the spinal cord [19,20]. An increased generation of superoxide may be a common link between at least three pathogenetic pathways that meet the prerequisites for forming a vicious circle.

Hypothesis

The data revealed about prostatitis, OxS and nociception enables to identify possible positive feedback pathways that can be responsible for the chronicity of pain, allodynia and hyperalgesia experienced by prostatitis patients. Pain signaling begins by activation of primary sensory afferents. Cell bodies of primary sensory afferents lie in the dorsal root ganglia, one process reaching into the periphery and another to the neurons in the dorsal horn of the spinal cord. A noxious stimulus opens the pain sensors coupled to ion channels (including TRPA1 and TRPV1), leading to influx of sodium and calcium into the cell, and subsequent depolarization of a primary sensory afferent [21]. Depolarization leads to the next stage of signal transmission, which is the release of glutamate into the synapses in the dorsal horn of spinal cord. Dorsal horn neurons accept the signal by ionotropic glutamate receptors. Of these, NMDAR (N-methyl D-aspartate receptor) and Ca²⁺-permeable AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) facilitate influx of calcium into the cell and into the mitochondria [22]. Entry of calcium into the mitochondria increases the superoxide (O₂⁻) output of mitochondria [23,19]. Unless metabolized by Mn-SOD (manganese superoxide dismutase), superoxide may generate more dangerous hydroperoxyl radicals (HO₂⁻) that are capable of initiating lipid peroxidation [24]. Therefore, peripheral stimuli (initial pain) can lead to spinal OxS (Fig. 1(1)). This is in agreement with animal experiments that have revealed analgesic properties of Mn-SOD, and that peripheral injury is a sufficient cause of spinal OxS. Mn-SOD and superoxide are relevant for prostatitis because prostatitis patients have elevated prevalence of Mn-SOD polymorphisms [25]. Moreover (Fig. 1(2)) it has been shown that spinal OxS is a sufficient cause of pain [20]. Therefore, spinal OxS can be a key link in pain-causing pathways. Three positive feedback pathways that stem from spinal OxS (lipid peroxidation due to uncompensated superoxide overproduction) are elaborated below.

The first pathway consists of glutathione depletion by electrophilic LPP. Electrophilic LPP readily react with glutathione. If the level of glutathione is low, then these electrophilic LPP are more likely to distort proteins and deoxyribonucleic acid (DNA) [26,13]. More importantly, an animal experiment has shown that glutathione loss in the spinal cord induces pain and that systemic or intrathecal administration of glutathione can reverse such induction of pain [27].

The second pathway of this positive feedback might be mediated by electrophilic LPP that can pass the cell membrane by passive diffusion [26]. Since electrophiles, including cyclopentene isoprostanes are ligands of TRPA1 [17,18], and since central processes of primary sensory afferents express TRPA1 [28–30], it is plausible that these compounds can contribute to or maintain [31] the pain signal that initiated the superoxide overproduction at the first place.

It has been shown that NGF (nerve growth factor) elevates expression of TRPA1 [28,32]. Association between pain and systemic OxS in the absence of overt inflammation (unpublished data) in one hand, and association between seminal plasma NGF levels and pain severity in prostatitis patients [33] on the other hand lead to a hypothesis that NGF supports the vicious circle mediated by electrophilic LPP by upregulating TRPA1 on the central processes of primary sensory afferents. The third pathway is mediated by LPP that are ligands of prostanoid receptors. These ligands include but are not limited to 8-isoprostanes [12] and prostaglandin F₂α (PGF₂α) [34]. Animal experiments have shown the ability of peripheral 8-isoprostanes and spinal PGF₂α to cause sensitisation [35,36]. Moreover, it has been shown that prostagland signaling is a necessary step that converts short-term signaling through NMDAR into long-term changes in neuronal phenotype [37]. This mechanism could provide additional explanation to the previously suggested idea that a sensitized state remains even after the initiating stimulus is lost [1]. A sensitized phenotype in prostatitis patients is evident, as prostatitis patients have increased sensitivity to cold, capsaicin (a TRPV1 agonist) and mechanical stimuli [38–40]. Activation of prostanoid receptors by isoprostanes that are generated by free radicals rather than by cyclooxygenase-2 can explain why cyclooxygenase-2 inhibitors have had conflicting results [5,6].

Highly electrophilic and bioactive cyclopentene isoprostanes are formed in human neural tissue by the same non-enzymatic pathway as 8-isoprostanes [13]. A generation of cyclopentene isoprostanes would not occur without the release of 8-isoprostanes into the system (Fig. 1(3)). Therefore, elevation of spinal 8-isoprostanes contributes to elevation of urinary 8-isoprostanes. Spinal OxS contributes to systemic OxS by competing for rate-limiting precursor of glutathione i.e. cysteine [41]. This is in agreement with our observation that in comparison with the controls, the blood level of glutathione is 15% lower in prostatitis patients (983 ± 57 μmol vs 1157 ± 73 μmol) [10].

Our observation also revealed that prostatitis patients’ lipid peroxidation markers 8-isoprostanes were elevated by 132% (5.8 ± 1.5 ng/ml vs 2.5 ± 0.9 ng/ml) [10]. The relatively stable 8-isoprostanes can affect lower urinary tract of rabbits in nanomolar concentrations [42]. This suggests that 8-isoprostanes could contribute to prostatitis symptoms by contracting bladder and urethra, which could cause lower urinary tract symptoms (LUTS), mainly urinary urgency and frequency (Fig. 1(4)). In this way, generation of 8-isoprostanes due to prostate inflammation or nervous OxS, and their accumulation into the urine can exert positive effects.
feedback by irritating the primary sensory afferents of bladder, prostate and urethra. Therefore, if the results of the rabbit study [42] apply to humans, then 8-isoprostanes are a valid cause of LUTS. At the same time, patients with asymptomatic inflammatory prostatitis have increased 8-isoprostane levels but no LUTS according to our earlier experiment [10] and newer unpublished data. Therefore, elevation of 8-isoprostanes is not a sufficient cause of LUTS. However, the level of 8-isoprostanes in urine might determine whether pre-existing urine reflexes, [43] anatomical deviations [44] or leaky urethra [45,46] remain subclinical or become symptomatic.

We propose that the chronic pelvic pain in prostatitis patients is caused by a vicious circle where LPP from dorsal horn of the spinal cord excites and sensitizes the primary sensory afferents. In addition, we propose that 8-isoprostanes that are secreted with urine may contribute to LUTS in vulnerable prostatitis patients, and that in patients with spinal OxS elevation of urinary 8-isoprostane levels corresponds partially to their spinal OxS.

**Evaluation of the hypothesis**

The limitation of the hypothesis is that a proportion of ‘non-bacterial’ prostatitis cases is probably caused by some bacteria that escape routine detection [47, 48], and are often combined with calcifications [49]. These mechanisms are independent from LPP-mediated positive feedback in the spinal cord or urinary tract. The relevance of spinal OxS for the pain can be tested by correlating pain intensity with spinal OxS and the ratio of spinal to systemic OxS. The relevance of spinal OxS pathway could be also tested by treating the patients with antioxidants that can pass through the blood–brain barrier and antagonize superoxide production in the mitochondria, controlled vs placebo, or as fluoroquinolone treatment combined with antioxidant treatment vs fluoroquinolone treatment without antioxidants. It can be also investigated whether antioxidants add value to a combination of fluoroquinolones, alpha-blockers and suitable COX inhibitors. However, tetracycline (or its newer analogs) is antibacterial against several relevant pathogens [50], can dissolve calcifications [51] and has shown superoxide antioxidation in *vitro* [52]. The relevance of urinary pathway can be tested by correlating LUTS with 8-isoprostanes in urine and in blood. 8-Isoprostanes have minor sensitizing properties and probably penetrate into the tissues more easily if the barrier function of the urinary epithelium is impaired. It cannot be assumed that 8-isoprostanes are functionally independent of TRPV1, TRPA1, impaired barrier function of urinary epithelium, spinal sensitisation, and anatomical deviations. The relevance of urinary pathway could be determined by measuring LUTS in the context of a treatment that reduces 8-isoprostanes in urine. If the LPP-mediated pathways exist, and are relevant for prostatitis, then there might be respective increase of interest, both clinical and scientific, towards antioxidants.

**Conflict of interest statement**

None declared.

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