

Received: 2014.04.22
Accepted: 2014.07.23
Published: 2014.12.02

Opening Tight Junctions may be Key to Opening the Blood–Prostate Barrier

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Data Interpretation D
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Source of support: Self financing

The blood-prostate barrier could be the major factor that preventing delivery of drugs to prostate tissue and leads to the failure of treatment. Studies indicate that ultrasonic sonoporation can break the blood-prostate barrier and increase the concentration of drugs, but the mechanism is still unclear. Tight junctions exist widely in the endothelial and epithelial cells of mammals, and form the biological barrier, along with other factors. Through studies on the mechanism of ultrasound microbubbles opening the blood-brain barrier, researchers found that the main mechanism is to change the expression level of TJs proteins. Since there might be some similarities between the blood-brain barrier and the blood-prostate barrier, changing the expression level of TJs proteins may also be the main mechanism by which ultrasound microbubbles opens the blood-prostate barrier, which is worth further study.

MeSH Keywords: **Claudins • Prostatitis • Tight Junctions**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/890902>

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Background

Chronic prostatitis is a common disease among men [1], and the incidence is gradually increasing. Due to the complicated condition of patients, the suboptimal effect of treatment, and the high rate of relapse, it seriously influences the physical and mental health of men. Although a many studies have been done on this topic, the pathogenesis and pathophysiological alterations and the best treatment are still unknown.

Recent studies have shown that the main problem of chronic prostatitis is that medicine cannot fully enter the prostate tissue, which limits the efficiency of the treatment. What is it that impedes the permeability of medicine into the prostate? Some researchers put forward the concept of a “blood-prostate barrier” [2]. Similar to the blood-brain barrier, the blood-prostate barrier protects the prostate tissue and has the function of permselectivity. In multicellular organisms, tight junctions (TJs) are intercellular junctions adjacent to the apical end of the lateral membrane surface [3]. They have a barrier function and a fence function. The changes of the TJs proteins can affect the blood-brain barrier permeability [4]. In studies on ultrasound microbubbles changing the blood-brain barrier, researchers found that the main mechanism of ultrasound microbubbles affecting the blood-brain barrier permeability is changing the expression level of TJs proteins. We hypothesize that this is also the main mechanism by which ultrasound microbubbles change the blood-prostate barrier permeability. At present, there are few relevant research reports and the topic deserves further study.

Hypothesis: Opening the Tight Junctions (TJs) may be Key Point to Enhancing Permeability of the Blood-Prostate Barrier

Tight junctions are intercellular junctions adjacent to the apical ends of paracellular spaces [3]. TJs are responsible for the regulation of transmembrane transport of ions and solutes (the fence function) and maintenance of cell polarity (the barrier function) [5]. Studies have shown that the TJs are ubiquitous in the blood-brain barrier [6], the enteric epithelial barrier [7], and the blood-testis barrier [8], and significantly regulate the barriers' functions. An experiment has shown that the TJs also exist in prostate tissue, but did not indicate the relationship between the TJs and the blood-prostate barrier [9]. A recent study has shown that ultrasound microbubbles may enhance blood-prostate barrier permeability through opening the TJs [10].

The tight junction is a fundamental junctional complex, composed of transmembrane proteins, cytosolic proteins, and cytoskeletal proteins. The transmembrane proteins include 3

kinds of integral membrane proteins: occludin [11], claudins [12], and junctional adhesive molecule (JAM) [13]. Occludin and claudin may play a primary role in regulating the barrier function and claudins are even more important [14].

The claudins are the essential component of TJs, and they are prevalent in the epithelia and endothelia. Some claudins create paracellular pores that are the basis for paracellular permeability, allowing small ions and molecules to pass through. However, others decrease the paracellular permeability [15]. This function is achieved by the protein kinase pathway – PKA or PKC acts on the specific amino acid sites of claudins. TJ function is decreased by closed phosphorylation [16], indicating that various physiological or pathological factors may influence the permeability of TJs by changing the expression level of the claudins. Furuse et al. identified claudin-1 and claudin-2 from chicken liver and found that over-expression of these 2 proteins also creates a tight junction between the fibroblasts [17], demonstrating their importance in the assembly of TJs. In recent years, many tests have indicated that claudins are very important to the blood-brain barrier. Morita et al. confirmed that there is rich expression of claudin-3 and claudin-5 in the brain microvascular endothelial circulation system and that claudin-5 is the major transmembrane protein of the blood-brain barrier [18]. A study using a C6 glioma model indicated that the dysfunction of TJs enhances the permeability of the blood-tumor barrier, probably by the direct invasion of C6 glioma cells, which reduces the expression level of claudin-5 in the endothelial cells [19]. It has been proposed that both over-expressing and ablating expression of claudins improve biofilm barrier permeability [20]. All of these studies suggest that it is possible to regulate biofilm barrier permeability by changing the expression level of claudins.

TJs exist in rat prostatic epithelial cells, and the expression levels of claudin-1,-3,-4,-5,-7,-8,-10 in the prostate are significantly higher than in the kidney [17]. It was previously demonstrated that claudin-1, -3, -4, -7 are expressed in rat and human prostate tissue [21], indicating that these claudins may play the same role in the rat and human prostate barrier. The barrier function of TJs may limit drugs permeability [22]. Therefore, it may be the key to treatment of chronic prostatitis, solving the problem of how to overcome the barrier properties of tight junctions and help move drugs into the prostate tissue.

Therefore, we suggest that: 1) Claudins are important regulators of the barrier function of TJs; and 2) Claudins not only expressed in the blood-brain barrier, but also in rat and human prostate tissue. We propose this hypothesis: Opening the tight junctions (TJs) may be a key to enhancing the permeability of the blood-prostate barrier. We plan to observe the expression of claudins in rat prostate to determine the changes occurring in blood-prostate permeability in future studies.

Discussion

As mentioned above, the blood-prostate barrier could be the major factor leading to the failure of treatment of chronic prostatitis, but the active component of the blood-prostate is still unknown. Some researchers consider that vascular endothelial cells and basement membrane comprise the barrier, but epithelial cells are deemed essential by others. It is certain that, similar to the blood-brain barrier, the blood-prostate barrier may be a key point in treatment of chronic prostatitis.

The ultrasonic contrast agent microbubble is a new kind of contrast agent; it not only enhances ultrasound imaging, but also increases the permeability of local organizations, thus improving the effective drug concentration [23,24]. Some researchers [10,25] have used ultrasound microbubble to enhance the blood-prostate barrier permeability, and indicated the possible mechanism – the ultrasound microbubble opens the tight junctions of vascular endothelial cells and epithelial cells of prostate tissue, then leads Evans blue (EB) dye into the glandular epithelium cells. Therefore, we consider that the tight junctions may be the main regulator of blood-prostate permeability.

To elucidate the molecular biology basis of the prostate tissue that limits the penetration of drugs into the prostate, and further understand the mechanism by which ultrasound microbubble enhances the prostate permeability, we plan to start with the expression level of tight junctions proteins, and observe changes in the expression of TJs proteins after the ultrasound microbubble acts on the prostate. This will show how the ultrasound microbubble affects the blood-prostate barrier. We propose to intravenously inject the ultrasound contrast agent microbubbles, and then make use of sonoporation induced by ultrasonic cavitation to open the blood-prostate barrier, and observe the relationship of the expression level of tight junction proteins and the level of the opening of the blood-prostate barrier. To achieve this aim, we will establish a rat model with CP in future studies, including a normal control group (NC group), chronic prostatitis model group (CP group), microbubble-only group (MB group), ultrasound-only group (US group), and microbubble-enhanced therapeutic ultrasound group (MEUS group, and different intensity ultrasound subgroups), and inject the microbubbles and Evans blue (EB), then observe the prostate tissue ultrastructure (e.g., vascular endothelial cells, basement membrane, basal cells, epithelial cells) after ultrasonic irradiation, and then test the concentration of EB in the prostate tissue by fluorescence spectrophotometry and analyze the expression level of the TJs proteins by immunohistochemistry and Western blot. Then we will make the following comparisons: 1) Compare the expression level of the TJ proteins between the NC group and CP group; 2) Compare the concentration of EB in the prostate tissue and the expression

level of the TJs proteins underlying the different intensity ultrasound; and 3) Compare the expression level of the TJs proteins between the MB group, US group, and the MEUS group. If the difference in expression level of TJ proteins in the NC group and CP group are statistically significant, it suggests that chronic inflammation could alter the blood-prostate barrier. If the degree of changes of the concentration of EB is consistent with the expression level of TJs proteins, then it indicates that the change of the blood-prostate is consistent with TJs. If the differences in expression level of the TJs proteins in the MB group, US group, and MEUS group are statistically significant, together with the result of the EB in the prostate tissue and the expression level of the TJs proteins underlying the different intensity ultrasound, it would suggest that the ultrasound microbubble enhances the permeability of the blood-prostate barrier by changing the expression level of the TJs proteins. Moreover, it is known that the TJs proteins could regulate the permeability of the TJs, which means the ultrasound microbubble opens the blood-prostate barrier by opening the tight junctions. We hope to prove our hypothesis that opening the tight junctions may be a key to enhancing the permeability of the blood-prostate barrier.

Conclusions

Chronic prostatitis is an intractable problem in urology. It is difficult to completely cure chronic prostatitis, mostly because the blood-prostate barrier limits the passage of drugs into the prostate tissue, which therefore fails to form an effective drug concentration in the prostate [25]. However, ultrasound microbubble technology can increase blood-prostate barrier permeability, and at the same time improve the effective drug concentration. This may become a new method for treatment of chronic prostatitis.

However, at present the mechanism of this method is still unclear. Therefore, we plan to start with the expression level of tight junctions proteins to explore the mechanism, and study it through the above experimental procedure and method, which may elucidate the mechanism of enhancing the blood-prostate barrier permeability and determine the composition of blood-prostate barrier. This will provide theoretical and methodological foundations to guide development of a new treatment for chronic prostatitis. At present, much research has been devoted to investigating tight junction protein regulation of the blood-brain barrier, but few have studied the blood-prostate barrier, which is a topic deserving further study.

Conflict of interest statement

None declared.

References:

1. A Khaikova, S Sohar, V Kazlouskaya: P3-S1.05 Incidence of STI in patients with chronic prostatitis. *Sex Transm Infect*, 2011; 87: A268
2. Fulmer BR, Turner TT: A blood–prostate barrier restricts cell and molecular movement across the rat ventral prostate epithelium. *J Urol*, 2000; 163: 1591–94
3. Sawada N, Murata M, Kikuchi K et al: Tight junctions and human diseases. *Med Electron Microsc*, 2003; 36: 147–56
4. Bénardais K, Pul R, Singh V et al: Effects of fumaric acid esters on blood–brain barrier tight junction proteins. *Neurosci Lett*, 2013; 555: 165–70
5. Sawada N: Tight junction-related human diseases. *Pathol Int*, 2013; 63: 1–12
6. Cioni C, Turlizzi E, Zanelli U et al: Expression of tight junction and drug efflux transporter proteins in an *in vitro* model of human blood–brain barrier. *Front Psychiatry*, 2012; 3: 47
7. Förster C: Tight junctions and the modulation of barrier function in disease. *Histochem Cell Biol*, 2008; 130: 55–70
8. Meng J: Androgens regulate the permeability of the blood–testis barrier. *Proc Natl Acad Sci USA*, 2005; 102(46): 16696–700
9. Sakai N, Chiba H, Fujita H et al: Expression patterns of claudin family of tight-junction proteins in the mouse prostate. *Histochem Cell Biol*, 2007; 127: 457–62
10. Liu Y, Yi S, Zhang J et al: Effect of microbubble-enhanced ultrasound on prostate permeability: A potential therapeutic method for prostate disease. *Urology*, 2013; 81: 921.e1–921.e7
11. Furuse M, Hirase T, Itoh M: Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol*, 1993; 123: 1777–88
12. Morita K, Furuse M: Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc Natl Acad Sci USA*, 1999; 96: 511–16
13. Aurrand-Lions M, Johnson-Leger C: Heterogeneity of endothelial junctions is reflected by differential expression and specific subcellular localization of the three JAM family members. *Blood*, 2001; 98: 3699–707
14. Oliveira SS, Morgado-Díaz JA: Claudins: multifunctional players in epithelial tight junctions and their role in cancer. *Cell Mol Life Sci*, 2007; 64(1): 17–28
15. Will C, Fromm M, Müller D: Claudin tight junction proteins: novel aspects in paracellular transport. *Perit Dial Int*, 2008; 28(6): 577–84
16. Fontijn RD, Volger OL, Fledderus JO et al: SOX-18 controls endothelial-specific claudin-5 gene expression and barrier function. *Am J Physiol Heart Circ Physiol*, 2008; 294(2Pt2): 891–900
17. Furuse M, Fujita K, Hiiragi T et al: Claudin-1 and -2: novel integral membrane Proteins localizing at tight junctions with no sequence similarity to occluding. *J Cell Biol*, 1998; 141(7): 1539–50
18. Morita K, Sasaki H, Furuse M et al: Endothelial-claudin: claudin-5 /TMVCF constitutes tight junction strands in endothelial cells. *J Cell Biol*, 1999; 147(1): 185–94
19. Rong Li R, Liu GL et al: Research on the C6 glioma in the rat blood-brain barrier ultrastructure and the changes of claudin-5. *Chinese Journal of Nerve Tumor*, 2008; 6(3): 184–88
20. Yu AS: Claudins and epithelial paracellular transport: the end of the beginning. *Curr Opin Nephrol Hypertens*, 2003; 12: 503–9
21. Swisshelm K, Robertson R, Kubbies M, Hosier S: SEMP1, a senescence-associated cDNA isolated from human mammary epithelial cells, is a member of an epithelial membrane protein superfamily. *Gene*, 1999; 226: 285–95
22. Chiba H, Osanai M, Murata M et al: Transmembrane proteins of tight junctions. *Biochim Biophys Acta*, 2008; 1778(3): 588–600
23. Skyba DM, Price RJ, Linka AZ et al: Direct *in vivo* visualization of intravascular destruction of microbubbles by ultrasound and its local effects on tissue. *Circulation*, 1998; 98(4): 290–93
24. Unger EC, Hersh E, Vannan M et al: Local drug and gene delivery through microbubble. *Prog Cardiovasc Dis*, 2001; 44(1): 45–54
25. Li T, Liu G, Li J, Wang X et al: Mechanisms of prostate permeability triggered by microbubble-mediated acoustic cavitation. *Cell Biochem Biophys*, 2012; 64(2): 147–53