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Effect of melatonin on contractile activity in intraurethrally instilled *E. coli*-induced acute prostatitis rat model

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Aim: To investigate whether prostatic inflammation affects the contractile responses of prostate tissue and then whether melatonin has any restorative effect on the contractile responses in a rat model of bacterial prostatitis at early stages.

Materials and methods: We evaluated the nerve-evoked, adrenergic agonist-induced or cholinergic agonist-induced contractions of isolated prostate tissue at 24 and 72 h after intraurethrally instilled *Escherichia coli*-treatment. We also analyzed the histological changes in the prostate. Secondarily, we investigated the effect of melatonin pretreatment (7 days, 10 mg kg^{-1} day^{-1}) on the contractile responses and the histopathologic changes.

Results: The degrees of acute inflammatory cell infiltration, acinar changes, and interstitial fibrosis in the prostate glands indicated the development of acute prostatitis at 24 or 72 h after bacterial inoculation. These inflammatory changes were more pronounced at 72 h. Bacterial prostatitis attenuated contractile responses to electrical field stimulation, phenylephrine, or carbachol. The impairment in the contractions was more prominent at 72 h. Melatonin treatment reduced the pathologic changes and partially restored the contractile responses at 72 h of inflammation.

Conclusion: Prostatic inflammation caused a diminution in the contractile mechanism of the prostate tissue and melatonin partially restored the contractile responses and histopathologic changes. Melatonin can be useful as an adjuvant to the main therapies for prostatitis to reduce the contractility problems.

Key words: Prostatitis, *Escherichia coli*, melatonin, smooth muscle contraction

1. Introduction

Bacterial prostatitis is a common problem in adult men and the perineal pain seen on ejaculation with prostatitis causes poor quality of life (1). It was suggested that the pain of prostatitis may be due to smooth muscle contraction (2,3). It is well known that prostatitis has an important role in the development of benign prostatic hyperplasia and prostate cancer (4,5).

Escherichia coli is the most common pathogen associated with both acute and chronic bacterial cystitis and prostatitis (6,7). Some recent papers showed that prostatitis elicited by *E. coli* caused an uncontrolled growth in the prostate gland at the early stages of inflammation (8,9). On the other hand, in previous studies, it was shown that uropathogenic *E. coli* or bacterial lipopolysaccharide (LPS) treatment causes hypocontractility in various tissues, including vessels, the urinary bladder, the corpus cavernosum, and the vas deferens (10–14). It might thus be expected that inflammation causes hypocontractility in prostate tissue. Although some papers showed the pathological and molecular features of acute prostatitis in animal models, none showed the contractile features of the prostate tissue (9,15,16).

The antioxidant and antiinflammatory effects of the pineal hormone melatonin (17,18) on tissue oxidative damage were well demonstrated in several studies on experimental models (19–23). Additionally, in our previous study, we showed that melatonin could prevent

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and restore the contractile impairment of the cavernous tissue in mice treated with bacterial LPS (12).

In the present study, to clarify the above issues, we investigated whether inflammation affects the contractile function of the prostate tissue and whether melatonin restores possible hypocontractility due to prostatitis in the tissue. Thus, we planned to evaluate the nerve-evoked, adrenergic agonist-induced or cholinergic agonist-induced contractions of isolated rat prostate tissue to investigate whether prostatic inflammation alters the contractile activity at early stages. Since it has been well documented that the intraurethral *E. coli* inoculation method induced bacterial prostatitis in animals (9,15,16), we examined the tissue responses at 24 and 72 h after intraurethrally instilled *E. coli* treatment. We also analyzed the histological changes in the prostate at 24 and 72 h after infection.

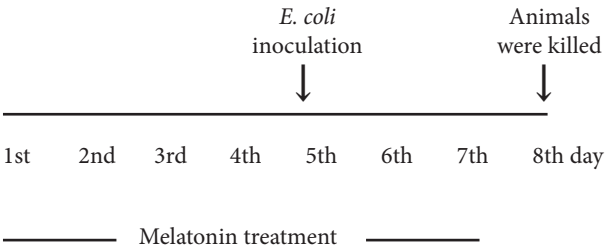
2. Materials and methods

2.1. Animals

Male Wistar albino adult rats, 3–4 months old and weighing 250–300 g (n = 50), were used throughout the experiments. The experimental procedure was approved by the Animal Care Committee of Çukurova University (TIBDAM; June 2009, 6/3) and the experiments were carried out in accordance with the Principles of Laboratory Animal Care (National Institute of Health guideline; Publication No. 86-23, revised 1984). All animals were kept under laboratory conditions (12 h dark; 12 h light) and allowed access to food and drink ad libitum.

2.2. Experimental groups

The rats (n = 50) were randomly divided into 4 groups: 1) the control group (n = 20), 2) the melatonin-treated group (n = 14), 3) the intraurethrally instilled *E. coli* treatment group (*E. coli*-treated group; n = 16), and 4) the *E. coli* + melatonin-treated group (n = 14). In the control groups, the vehicle, saline (0.2 mL), was instilled into the prostatic urethra of all animals (n = 16) and then the rats were killed 24 h (n = 8) or 72 h (n = 8) after this application. In the melatonin-treated groups, rats were treated with an intraperitoneal melatonin (10 mg kg^{-1} day^{-1} every day at 0900 hours; n = 8) or vehicle (n = 6) for 7 days. In these groups, saline (0.2 mL) was intraurethrally instilled on day 5 of melatonin treatment and then the animals were killed 72 h after saline inoculation on day 8. In the *E. coli*-treated groups, the rats were killed 24 h (n = 8) or 72 h (n = 8) after *E. coli* inoculation. In the *E. coli* + melatonin-treated groups, rats (n = 14) received melatonin (10 mg kg^{-1}) injections for 7 days, similar to the protocol mentioned above, and *E. coli* was intraurethrally instilled on day 5 of melatonin treatment and then the animals were killed 72 h after *E. coli* inoculation on day 8. The following schematic illustrates the timing these treatments graphically.



2.3. Drugs

Stock solutions of carbachol, phenylephrine, and KCl were dissolved in distilled water. Melatonin was dissolved in sterile saline. All drugs were obtained from Sigma Chemical Co., St. Louis, MO, USA.

2.4. Preparation of bacterial suspension (*E. coli*)

Bacterial suspensions were prepared in a microbiology laboratory. A strain of uropathogenic *E. coli*, isolated from patients with complicated urinary tract infections, was stored at 20 °C and grown overnight in a stock medium. Brain heart infusion (BHI) broth with 10% sheep blood and 10% glycerol was used as a stock medium in this study. This medium is used for culturing of uropathogenic strains. For inoculation to mice, we passaged strains from this BHI stock medium to a sheep blood agar. The next day we prepared a suspension with SF with a concentration of McFarland 3.

2.5. Bacterial prostatitis model

The rats were anaesthetized with sevoflurane and catheterized with a lubricated sterile polyethylene tube (Clay Adams, PE 10). An insulin syringe was attached to the needle and 0.2 mL of the bacterial suspension containing 9×10^8 colony-forming units/mL of *E. coli* was instilled into the prostatic urethra. Anesthesia was maintained for 1 h to prevent urinary leakage by movement of the rat and to allow a sufficient time for bacteria to invade the prostate (22). Animals were sacrificed at 24 or 72 h after bacterial inoculation for in vitro prostate tissue experiments and histopathological studies. The urine was collected before the animals were killed for microbiological studies.

2.6. Microbiological studies

For bacterial culture, urine was passaged into Endo Agar plates and incubated overnight at 37 °C. The colonies were evaluated in a suspension with saline at a concentration of McFarland 4 for uropathogenic *E. coli*.

2.7. Histopathological studies

Random samples of the ventral prostate from each group were fixed in 10% formalin for 3–6 h, dehydrated, embedded in paraffin, sectioned at 5 μm, and subsequently stained with hematoxylin and eosin. The slides were examined by light microscopy. The severity of prostatic inflammation was evaluated based on 3 parameters (the amount of chronic inflammatory cell infiltrates, acinar changes, and interstitial fibrosis) in the representative

area by pathologists who had no information on the experimental group.

2.8. Measurement of prostate smooth muscle contractile activity

Rats were killed by cervical dislocation under sevoflurane anesthesia. After the abdominal incision, the left and right lobes of the ventral prostate gland were removed and placed in a petri dish containing Krebs solution (mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$, $NaHPO_4$ 1.2, glucose 11). The capsules of the ventral prostatic lobes were removed along with connective and adipose tissues. Two preparations were obtained from each lobe. The prostate tissues were mounted under 1 × *g* tension between 2 platinum electrodes embedded in Perspex in 5-mL jacketed organ baths containing Krebs solution. The organ baths maintained 37 °C and aerated with a mixture of 95% O_2 and 5% CO_2. Tissues were then allowed to equilibrate for 1 h and were washed with fresh Krebs solution every 15 min during this period. Tissue responses were recorded via isometric force transducer (MAY, FDT 10-A). Data were recorded and stored using data acquisition software (MP30, BIOPAC Systems, Inc., Goleta, CA, USA). After the equilibrium period, tissues were contracted with 1) electrical field stimulation (EFS; 1, 2, 4, 8, 16, and 32 Hz; 50 V; 0.5-ms duration) delivered from a Grass S88 stimulator (Grass Instruments, Quincy, MA, USA) for 15 s at 1-min intervals; 2) an α_1-adrenergic receptor agonist, phenylephrine (0.1, 0.5, 1, 5, 10, 50, and 100 μM); or 3) a nonspecific cholinergic receptor agonist, carbachol (0.1, 0.5, 1, 5, 10, 50, and 100 μM). Phenylephrine or carbachol was added to the organ bath cumulatively. After each treatment, tissues were washed with fresh Krebs solution and kept for 30 min until the next application. At the end of the experiment, tissues were contracted with KCl (100 mM). After the contraction reached a steady state, the tissues were washed with Krebs solution.

2.9. Statistical analysis

Changes in tension induced by EFS, phenylephrine, or carbachol were expressed as the percentage (%) of the maximal response to 100 mM KCl-induced contraction at the end of each experiment. All data were expressed as mean ± SE and were analyzed by Student's t-test using GraphPad Prism software (San Diego, CA, USA). $P < 0.05$ was considered significant.

3. Results

3.1. Microbiological data of the urine cultures

There was no bacterial growth in the urine culture obtained from the control group. However, the urine culture showed bacterial growth in the *E. coli*-treated group. No bacterial growth was observed in melatonin + *E. coli*-treated rats (data not shown).

3.2. Histopathological data of the prostate

In control animals inoculated with saline, no inflammatory infiltrate was observed in the stroma of the ventral prostate gland (data not shown). However, macroscopic examination of *E. coli*-treated rats revealed markedly enlarged prostates with hyperemia and edema. The degrees of acute inflammatory cell infiltration, acinar changes, and interstitial fibrosis in the prostate glands indicated the development of acute prostatitis at 24 or 72 h after bacterial inoculation (Figures 1, 2A, and 2B). There was some infiltration by neutrophils in the stromal connective

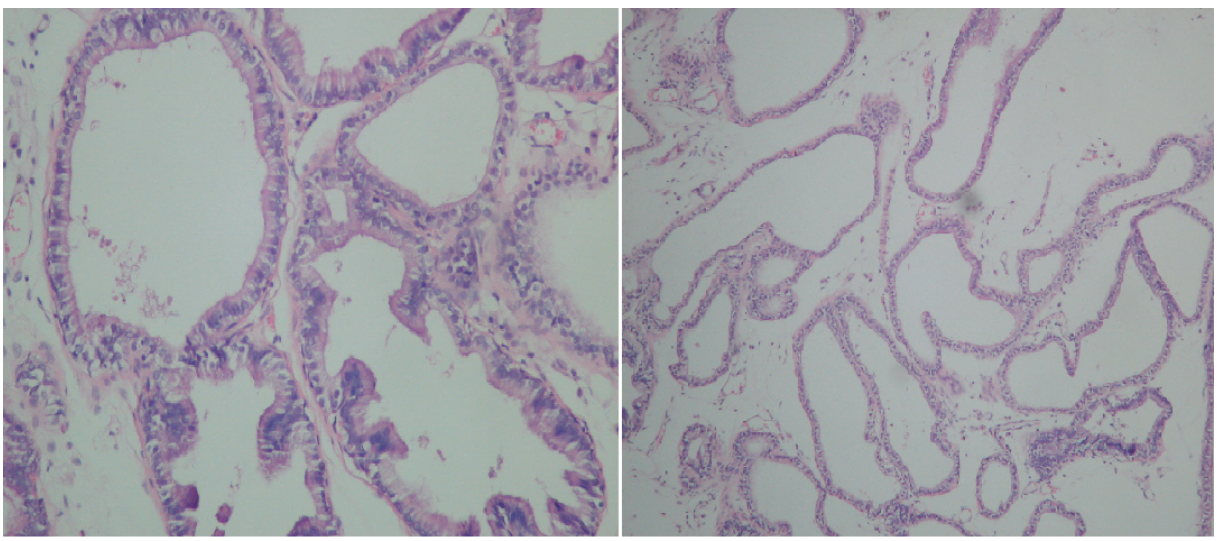


Figure 1. Prostatic section of acute bacterial prostatitis rats, obtained 24 h after treatment. The figures show a mild-to-moderate infiltration of acute inflammatory cells (hematoxylin and eosin, optical microscopy).

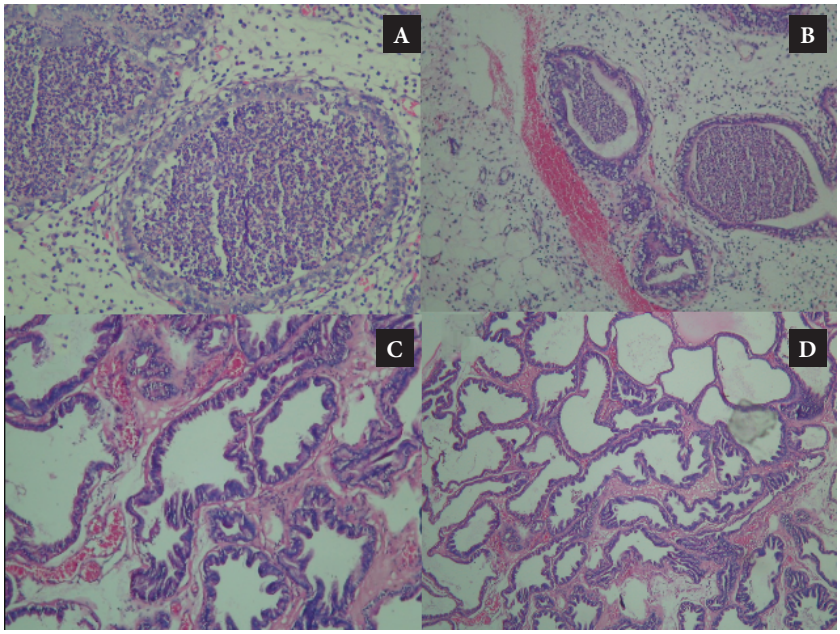


Figure 2. Prostatic section of acute bacterial prostatitis rats, obtained 72 h after treatment. A and B: severe infiltration by neutrophils in the stromal connective tissue around the acini or ducts in the prostate gland obtained 72 h after *E. coli* treatment. C and D: inflammatory cell infiltration and acinar changes decreased in the prostate gland obtained from melatonin + *E. coli*-treated group (n = 2) (hematoxylin and eosin, optical microscopy).

tissue around the acini or ducts in the prostate glands at 24 h after *E. coli* treatment (Figure 1). These inflammatory changes were more pronounced at 72 h of infection, with intense edema, reduction of the glandular lumen, and a great number of inflammatory cells invading the glandular compartment (Figures 2A and 2B). On the other hand, in the macroscopic examination of prostate glands, melatonin treatment significantly decreased the hyperemia and edema at 72 h of infection. A significant reduction in the inflammatory cell infiltration and acinar changes was also observed at 72 h after bacterial inoculation in the melatonin-treated group (Figures 2C and 2D).

3.3. Contractile activity in prostate smooth muscle strips obtained from control rats

EFS (1–32 Hz) induced frequency-dependent contractions in rat prostate smooth muscle. Phenylephrine (0.1–100 μM) or carbachol (0.1–100 μM) also contracted the prostate smooth muscle in a concentration-dependent manner. The contractions to these drugs were reversible and reproducible. However, cholinergic agonist-induced contractions were smaller compared to phenylephrine-induced contractions

3.4. Effects of *E. coli* treatment on contractions elicited by EFS in prostate smooth muscle strips

The contractile responses to EFS (1–32 Hz) were significantly decreased compared to those of controls at 24 or 72 h after *E. coli* treatment (Tables 1 and 2). However, this decrease was significant at only low frequencies ($P < 0.05$ at 1, 2, and 4 Hz) at 24 h (Table 1). On the other hand, at 72 h, the impairment in the contractile responses to EFS was more pronounced at all frequencies ($P < 0.05$; Table 2). Melatonin treatment (10 mg kg^{-1} day^{-1}) significantly restored the impairment in the contractions induced by EFS of the prostate smooth muscle, except that at the highest frequency (32 Hz) ($P < 0.05$; Table 2), at 72 h after bacterial inoculation. Treatment with the melatonin alone as used in control group did not cause any significant alteration in the response of the tissue to EFS (data not shown). The vehicle of melatonin used intraperitoneally in the *E. coli*-treated group also had no effect (data not shown).

3.5. Effects of *E. coli* treatment on contractions elicited by phenylephrine in prostate smooth muscle strips

The contractile responses to phenylephrine were significantly decreased compared to those of controls at 24 or 72 h after *E. coli* treatment (Tables 1 and 2). However, this decrease was significant at only low frequencies ($P < 0.05$ at 0.1, 0.5, and 1 μM) at 24 h (Table 1). On the other hand, at 72 h, the impairment in the contractile responses to phenylephrine was more pronounced at all concentrations ($P < 0.05$; Table 2). Melatonin (10 mg kg^{-1} day^{-1}) could significantly reverse this inhibition, except at

Table 1. The contractile responses to electrical field stimulation (1–32 Hz, 50 V, 0.5 ms), phenylephrine (0.1–100 μM), or carbachol (0.1–100 μM) in the prostate tissues obtained from control or *E. coli*-treated groups at 24 h.

Electrical field stimulation (Hz)						
	1	2	4	8	16	32
Control	14.0 ± 1.2	28.3 ± 2.2	49.8 ± 2.5	61.0 ± 1.5	77.3 ± 7.1	77.8 ± 3.2
E. coli	7.20 ± 0.4*	15.7 ± 2.1*	27.7 ± 3.2*	50.8 ± 4.8	58.8 ± 6.0	61.6 ± 5.6
Phenylephrine (μM)						
	0.1	0.5	1	5	10	50	100
Control	12.9 ± 2.1	34.9 ± 4.3	54.1 ± 6.2	83.7 ± 7.9	100.1 ± 9.9	102.8 ± 10.2	102.3 ± 8.8
E. coli	2.97 ± 0.3*	11.7 ± 3.4*	35.4 ± 3.8*	57.2 ± 7.0	69.2 ± 5.6	84.1 ± 4.5	84.9 ± 5.2
Carbachol (μM)						
	0.1	0.5	1	5	10	50	100
Control	8.20 ± 2.4	15.8 ± 2.9	25.5 ± 1.8	40.0 ± 4.1	48.1 ± 6.0	55.9 ± 7.6	57.4 ± 6.9
E. coli	3.22 ± 0.6*	7.3 ± 2.7	20.7 ± 1.3	29.9 ± 6.0	34.8 ± 7.2	36.9 ± 6.8	37.6 ± 9.7

Data represent the mean contractile response expressed as percentage of 100 mM KCl-induced contraction. Asterisk indicates significant differences in contraction between *E. coli*-treated and control groups at $P < 0.05$.

Table 2. The contractile responses to electrical field stimulation (1–32 Hz, 50 V, 0.5 ms), phenylephrine (0.1–100 μM), or carbachol (0.1–100 μM) in the prostate tissues obtained from control, *E. coli*-treated, or *E. coli* + melatonin-treated (*E. coli*-M) groups at 72 h.

Electrical field stimulation (Hz)						
	1	2	4	8	16	32
Control	31.8 ± 1.6	50.2 ± 2.3	81.0 ± 5.8	120.5 ± 11.0	141.1 ± 11.5	153.0 ± 10.4
E. coli	19.8 ± 2.5*	32.6 ± 3.3*	44.4 ± 6.6*	51.4 ± 8.5*	59.2 ± 8.1*	67.6 ± 10.4*
E. coli-M	33.5 ± 2.3+	50.0 ± 5.1+	73.5 ± 5.0+	85.5 ± 4.6+	96.5 ± 2.5+	108.7 ± 5.9+
Phenylephrine (μM)						
	0.1	0.5	1	5	10	50	100
Control	10.9 ± 1.9	37.2 ± 4.4	61.5 ± 6.6	91.8 ± 8.6	130 ± 11	150 ± 20	146 ± 14
E. coli	5.6 ± 0.8*	16.8 ± 2.6*	41.0 ± 3.7*	50.7 ± 5.8*	62.8 ± 2.9*	76.5 ± 9.6*	78.8 ± 9.2*
E. coli-M	14.2 ± 1.8+	41.8 ± 3.2+	59.0 ± 3.3+	77.3 ± 2.3+	88.3 ± 3.9+	95.8 ± 5.9+	101 ± 7.0+
Carbachol (μM)						
	0.1	0.5	1	5	10	50	100
Control	12.0 ± 2.9	18.4 ± 3.5	30.0 ± 5.6	50.8 ± 12	62.2 ± 13	68.8 ± 12	69.7 ± 11
E. coli	3.6 ± 0.0*	11.9 ± 4.8	20.3 ± 5.1	27.4 ± 8.3	32.1 ± 8.9	36.9 ± 8.5	40.5 ± 7.2
E. coli-M	5.0 ± 0.7	12.3 ± 2.3	16.3 ± 2.9	30.0 ± 5.6	38.8 ± 7.3	42.5 ± 7.3	46.9 ± 8.0

Data represent the mean contractile response expressed as percentage of 100 mM KCl-induced contraction. Asterisk indicates significant differences in contraction between *E. coli*-treated and control groups at $P < 0.05$. (+) indicates significant differences in contraction between *E. coli*-treated and *E. coli*-M groups for each column at $P < 0.05$.

high concentrations (50 and 100 μM) of phenylephrine, at 72 h after bacterial inoculation (Table 2). Treatment with the melatonin alone used in the control group did not cause any significant alteration in the response of the tissue to phenylephrine (data not shown). The vehicle of melatonin used intraperitoneally in the *E. coli*-treated group also had no effect (data not shown).

3.6. Effects of *E. coli* treatment on contractions elicited by carbachol in prostate smooth muscle strips

E. coli treatment caused some decrease in the contractile response of the tissue to carbachol at 24 or 72 h after *E. coli* treatment, but a significant impairment was observed at only the lowest concentration of carbachol at 72 h after bacterial inoculation ($P < 0.05$ at 0.1 μM; Tables 1 and 2). Melatonin (10 mg kg^{-1} day^{-1}) could reverse this inhibition (Table 2). Treatment with the melatonin alone used in the control group did not cause any significant alteration in the response of the tissue to carbachol (data not shown). The vehicle of melatonin used intraperitoneally in the *E. coli*-treated group also had no effect (data not shown).

4. Discussion

The major finding of the present study was that the bacterial prostatic inflammation elicited by intraurethrally instilled *E. coli* affected contractile activity of the rat prostate tissue at early stages. Prostatitis caused a marked impairment in the contractile activity induced by EFS, phenylephrine, or carbachol in rat prostate tissue at 24 or 72 h after *E. coli* treatment. Treatment with melatonin partially prevented the impairment in EFS-, phenylephrine-, or carbachol-induced contractile responses of the prostate gland in prostatitis rat. Melatonin treatment also significantly reduced the inflammatory cell infiltration and acinar changes at 72 h after bacterial inoculation.

In the present study, intraurethrally instilled *E. coli* caused acute inflammatory cell infiltration, acinar changes, and interstitial fibrosis in the prostate glands at 24 or 72 h after bacterial inoculation. These histopathologic features at early stages indicated development of an acute bacterial prostatic inflammation. These inflammatory changes were more pronounced at 72 h of infection, with intense edema, reduction of the glandular lumen, and a great number of inflammatory cells invading the glandular compartment. Our histopathologic findings were consistent with those of previous studies (9,15,16).

Bacterial prostatitis also significantly attenuated contractile responses of the prostate gland to electrical field stimulation (EFS), phenylephrine, or carbachol compared to those of control group. However, the impairment in the contractions was more prominent at 72 h of prostatic inflammation. This finding suggests that the impairment in the contractile mechanism of the tissue may be associated with the degree of prostatic inflammation since there was a severe infiltration in the prostate glands at 72 h after bacterial inoculation. This impairment of the contractile activity may be due to various reasons. It was shown that prostatic inflammation, even at the early stages, causes a dedifferentiation of smooth muscle cells (9). It was suggested in the same study that prostatic smooth muscle cells become secretory cells in response to *E. coli*. Additionally, Leimgruber et al. described a dedifferentiation process of smooth muscle cells after bacterial LPS treatment, with a loss of cytoskeleton molecules that characterized contractile phenotype (8). In another recent study, it was shown that inflammation caused the loss of smooth muscle cells around prostate ducts (24). Although we could not comprehensively examine the smooth muscles by light microscopy, it can be suggested that *E. coli* treatment affected the smooth muscles in the prostate gland in light of these papers.

However, some previous studies suggested that inflammation induced by uropathogenic *E. coli* or bacterial LPS alters the contractile mechanism of the tissues via production of free radicals and/or lipid peroxidation caused by the inducible nitric oxide synthase (iNOS)-related pathway (12,25–27). Therefore, it is possible that increased oxidative products due to prostatic inflammation induced by intraurethral *E. coli* application cause an impairment in the contractile mechanism of the prostate tissue. However, we need further experiments, such as studies of iNOS expression, to explain the impairment of the contractions with prostatic inflammation.

Interestingly, in some clinical studies with patients, the pain is thought to be due to prolonged smooth muscle contraction caused by α_1-adrenoceptor activation in the prostate (2,3,28). In these papers, it was suggested that α_1-adrenoceptor blockers might be useful, possibly by promoting smooth muscle relaxation in the treatment of prostatic pain. However, our findings indicate that bacterial inflammation causes a decrease in the contractile activity of the prostate gland. Thus, the beneficial effect of α_1-adrenoceptor blockers on the pain may be due to other mechanism(s) in prostatitis.

On the other hand, the impairment of the carbachol-induced contractions was less pronounced in the prostate tissue after *E. coli* treatment. However, cholinergic agonist-induced contractions were smaller compared to adrenergic agonist-induced contractions. Since it was shown that the contractile response to cholinomimetic agents such as acetylcholine or carbachol was less than that to adrenoceptor agonists (29), the contractions to carbachol might be less affected by prostatic inflammation.

In the present study, histopathologic results showed that melatonin treatment was partially effective to prevent severe infiltration in the prostate glands. Melatonin could also reverse the impairment of the neurogenic

and adrenergic contractions of prostate tissue. Beneficial effects of the pineal hormone melatonin on tissue oxidative damage have been demonstrated in studies on experimental models. In these studies, it was shown that melatonin has highly potent free radical scavenging ability and it was suggested that this action may contribute to the antiinflammatory effects of this agent (12,19–23). However, this possible mechanism must be supported by measuring antioxidant enzyme activities.

In conclusion, prostatitis caused a marked impairment in the neurogenic and adrenergic contractile activity in the rat prostate tissue. A possible dedifferentiation of smooth muscle cells and increased oxidative activity in the prostate tissue may be major reasons for the diminution in contractile mechanism. Treatment with melatonin prevented the impairment of the contractile responses and histopathologic changes due to prostatic inflammation. Melatonin can be useful as an adjuvant to the main therapies for prostatitis to reduce the contractility problems.

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