Changes of gap junctional cell-cell communication in overactive detrusor in rats

Longkun Li,1 Chonghe Jiang,2 Ping Hao,3 Weibing Li,1 Caiping Song,1 and Bo Song1
1Center of Urology, Southwest Hospital, Third Military Medical University, Chongqing, China; 2Department of Biomedicine and Surgery, University of Linköping, Linköping, Sweden; and 3Center of Oncology, Xinqiao Hospital, Third Military Medical University, Chongqing, China

Submitted 29 March 2007; accepted in final form 29 August 2007

Li L, Jiang C, Hao P, Li W, Song C, Song B. Changes of gap junctional cell-cell communication in overactive detrusor in rats. Am J Physiol Cell Physiol 293: C1627–C1635, 2007.—To evaluate the changes in intercellular communication through gap junctions in detrusor overactivity (DO), we studied 23 adult female Wistar rats with DO after partial outflow obstruction (DO group) and 13 sham-operated rats (control group). The two groups were compared by means of urodynamics, light and electron microscopy, expression of Cx40, Cx43, and Cx45 mRNA genes with RT-PCR, Cx43 protein with Western blot analysis, and functional intercellular communication with scrape loading dye transfer (SLDT) and fluorescence recovery after photobleaching (FRAP). The number of gap junctions and the expression of connexin mRNA and Cx43 protein were increased in DO rats, and intercellular communication through gap junctions increased after 6 wk of partial outflow obstruction as assessed with SLDT and FRAP techniques. The findings provide a theoretical rationale for using Cx43 antagonists and gap junction inhibitors in the treatment of patients with overactive detrusor secondary to partial bladder outflow obstruction.

Connexins are the principal protein component of the GJ, responsible for the functional generation of GJ channels. There are 12 different subunits in the connexin family, and their diversity is still poorly understood. Several connexins, including Cx40, Cx43, and Cx45, are confirmed to be in the detrusor myocytes (22), and Cx43 is the main one (23). These connexins are assumed to be essential for the cell-cell communication observed.

Detrusor overactivity (DO) is a common clinical problem in which the bladder has a higher excitability than normal. One of the main causes is partial bladder outflow obstruction (PBOO), in which overactivity could result neurogenically and/or myogenically from changes in the detrusor innervation and myocyte excitability. Recently, changes in cell-cell communication have been proposed to be one of the possible mechanisms (3, 8), and Cx43 plays an important role in the development of DO (6, 25). However, the functional changes of cell connectivity in DO are not well elucidated, and there is considerable controversy between reports on changes in cell-cell contact in DO after PBOO. Christ et al. (7) showed a 75-fold increase of Cx43 mRNA expression compared with the control in DO cells in rats after 6 wk of PBOO, whereas others using scrape loading dye transfer (SLDT) found an increase in dye transfer in the bladder urothelial cells of the rats with acute bladder outflow obstruction. Mori et al. (21) revealed an initial increase in Cx43 mRNA expression, but this was followed by a secondary decrease. The inconsistencies might be due to variations in the time course of the PBOO or the animal model used.

To date, there are few other reports about cellular connectivity in DO myocytes. The goals of the present study were to clarify the changes in function in the GJ of detrusor myocytes and their role on the development of DO following PBOO by using several experimental techniques in a rat model. We used RT-PCR to evaluate the expression of Cx43, Cx45, and Cx40 mRNA, Western blotting to half-quantitatively analyze the Cx43 protein, and SLDT and fluorescence recovery after photobleaching (FRAP) to determine the functionality of the GJ channels. The outcomes of all these experimental approaches showed an increase in the expression and functions of connexins mediating intercellular communication in the overactive detrusor.

MATERIALS AND METHODS

Forty-three adult female Wistar rats weighing 180–220 g were used in this study. The animals were housed in mesh-bottom cages

SMOOTH MUSCLE from the urinary bladder displays characteristic and consistent patterns of contractile activity during micturition due to the common innervation and mutual communication between the cells. The ability of the bladder to function normally depends on the correct behavior of several cell types, but the cellular activity of all the smooth muscle cells must be coordinated with each other. This consistency could come through long-distance modulation mediated by neural or endocrine mechanisms or through short-distance interactions such as cell-cell transmission. Studies have shown that detrusor myocytes can contract synchronously even in isolated bladders in vitro, which emphasizes the importance of the efficacy of cell-cell contacts between the detrusor myocytes (3).

Signals between cells can be transferred through electrical and metabolic coupling. Gap junctions (GJ), specialized cell surface membrane channels connecting neighboring cells for cell communication, provide low-resistance pathways between the cells, which permit passive diffusion of molecules with apparent selectivity for those smaller than 1 kDa, such as ions and the second messengers cAMP and cGMP. Thus intercellular communication through GJs is important for sustaining and transferring excitation between cells (16, 26).
with free access to food and water ad libitum and were maintained on a light-dark cycle at 22–24°C. During experiments in vivo, the animals were kept under a heat lamp to prevent a decrease in body temperature, and they were killed by overdose of anesthetics. All experimental protocols were approved by the Animal Research Ethics Committee of the Third Military Medical University.

Preparation of PBOO models. Thirty Wistar rats were anesthetized by intraperitoneal injection of phenobarbital (40 mg/kg). PBOO was created as reported previously by our group (18). In brief, the urinary bladder was catheterized with a small plastic tube (1.0-mm outer diameter) via the urethral orifice. After a low abdominal incision was made, the bladder neck was tied around the catheter using a 2/0 silk ligature. The tube was then removed, and the incision was closed. Thirteen rats underwent a sham operation using the same procedure except for tying the ligature. All the animals received prophylactic antibiotics after the operation by intraperitoneal injection of 20,000 units of penicillin, followed by oral administration of 400 mg/l oxytetracycline hydrochloride three times a day for 3 days.

Cystometry and group classification. Cystometry was performed after 6 wk of PBOO. Rats were anesthetized by subcutaneous injection of urethane (1 g/kg). With the ligature for PBOO still in place, the bladder was catheterized through the urethra by a human epidural catheter (2-F internal diameter), which was connected to urodynamic equipment (Dantec Menuet, Skovlunde, Denmark) via a three-way connector for both infusion and pressure recording. Cystometry was performed by infusing warm saline (37–38°C) at a rate of 0.2 ml/min, and the infusion was stopped when leakage of urine was seen around the catheter. Bladder emptying was ascertained by opening the catheter and gently pressing the lower abdomen after each trial. Three consecutive cystometries were performed in each animal at intervals of 30 min to show consistent bladder behavior. During bladder filling, some PBOO animals had obvious nonvoiding detrusor contractions before the onset of micturition and thus were defined as having DO and classified as the DO group (n = 23) (1, 9). The other seven PBOO rats, with a stable detrusor before the onset of micturition contractions, were excluded from the present study. The 13 sham-operated rats were classified as the control group.

Histology. The rats were killed by overdose of urethane, and a vertical strip from the bladder tissue was obtained and fixed with 10% formalin. After ethanol gradient dehydration and paraffin embedding, slices were cut, stained with hematoxylin and eosin, and examined under light microscopy. Another similar bladder strip was fixed with

### Table 2. Urodynamic results of control and DO groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual urine, ml</td>
<td>0.1±0.01</td>
<td>2.8±0.51†</td>
</tr>
<tr>
<td>Bladder capacity, ml</td>
<td>0.9±0.08</td>
<td>3.7±0.62†</td>
</tr>
<tr>
<td>Bladder compliance, ml/cmH₂O</td>
<td>0.19±0.04</td>
<td>0.12±0.03*</td>
</tr>
<tr>
<td>Threshold pressure for voiding, cmH₂O</td>
<td>10.8±2.43</td>
<td>15.3±3.22*</td>
</tr>
</tbody>
</table>

DO, detrusor overactivity. *P < 0.05; †P < 0.01 vs. control.
3% glutaric dialdehyde and then with 2% perosmic acid. After ethanol gradient dehydration, strips were embedded in Epon812 ethoxyline resin, slices were cut (50 nm), stained with uranyl acetate-lead citrate, and evaluated with electron microscopy (Philips CM10; Eindhoven, The Netherlands).

**Analysis of mRNA encoding Cx40, Cx43, and Cx45.** After careful removal of bladder mucosa and urothelium under a dissecting microscope, detrusor tissue was taken (100 mg) for RNA extraction. The tissue was chopped into small pieces, and the total RNA was treated with DNase. RNA concentration and quality were determined by agarose gel electrophoresis and ultraviolet spectrophotometry. RNA with a ratio of absorption at 260 and 280 nm ($A_{260}/A_{280}$) above 1.8 was used for the study. mRNAs encoding Cx40, Cx43, and Cx45 were detected by RT-PCR. TaqDNA polymerase, AMV reverse transcriptase, oligo(dT), dNTP, RNase inhibitor, PCR Marker (Promega), TriPure isolation reagent (Roche), and diethylmaleate pyrophosphoric acid (Sigma) were used for RT-PCR assay. Primers for Ca, SKCa2, and SKCa3 channels were designed with DNAStar software. All the primers, verified with the Basic Local Alignment Search Tool (BLAST), were synthesized by Sangon (Shanghai, China), and their sequences are shown in Table 1. With an internal control of $\beta$-actin, RNA was reverse-transcribed and amplified by application of the RT-PCR kit (Promega). The first-strand cDNA was synthesized at 42°C for 60 min and then heated at 95°C for 10 min to terminate the cDNA synthesis reaction. Bladder cDNA for specific primers was amplified for 35 cycles by PCR at 94°C for 90 s, 56°C for 60 s, and 72°C for 60 s and then extended at 72°C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide, and photographed. Optical density was assessed from the photograph with an image analyzer and was normalized to the internal control of $\beta$-actin. All the experimental protocols were performed in triplicate.

**Western blot analysis.** The urothelium-denuded bladder smooth muscle (100 mg) was prepared in buffer (pH 7.2) containing 1 mM Tris-HCl, 1 mM DTT, 2% SDS, and 0.1% bromchlorphenol blue. Cells were dispersed by ultrasound and centrifuged at 1,000 rpm for 10 min at 4°C, and then the pellets obtained were incubated in homogenization buffer with 1 mM phenylmethylsulfonyl fluoride at −20°C. Protein concentrations were determined using the Bio-Rad machine and subjected to SDS-PAGE on 12% gels. Proteins were transferred to nitrocellulose membranes by electrobloating, and membranes were incubated with mouse anti-Cx43 antibody (1:500; Sigma)
at 4°C overnight, followed by incubation with horseradish peroxidase-conjugated anti-mouse IgG (1:500; Sigma) for 1 h at 37°C. Blots were developed using the DAB Western blotting detection reagent (Bode Bioengineering, Shanghai, China) and scanned to identify the integration optical density with a Bio-Rad image analyzer.

**Preparation of cultured detrusor cells.** The remaining detrusor tissue was incubated in HEPES solution (HEPES) containing (in mM) 19.5 HEPES, 105.4 sodium chloride, 22.3 sodium hydrogen carbonate, 3.6 potassium chloride, 0.9 magnesium chloride hexahydrate, 0.4 monosodium orthophosphate dihydrate, 5.4 glucose, and 4.5 sodium pyruvate, and the pH of the solution was adjusted to 7.1 with sodium hydroxide. The detrusor strips were digested overnight at 4°C in HEPES with 0.1% type IV collagenase but no calcium, and cells were dispersed. The cells were then rinsed in HEPES three times and transferred into Dulbecco’s modified Eagle’s medium (DMEM) solution containing 1.34% DMEM, 0.3% sodium bicarbonate, 0.05% glutamine, 0.15% HEPES, 100 U/ml penicillin, 100 U/ml phytohemagglutinin, and 15% fetal bovine serum for culture at a temperature of 37°C and bubbling with 5% CO₂. Only P0 (dispersed and cultured but never passaged) cells were used.

Analysis of GJ connectivity with SLDT. To determine cellular connectivity with SLDT, cultured cells at 90% confluence were rinsed three times with PBS. A scalpel was used to create a cross scrape through the monolayer in the extracellular solution with 0.5% hydrophilic dye Lucifer yellow. After 3 min of incubation in a dark room at room temperature, the culture was rinsed again three times with PBS (10). Then cells were then fixed with 4% paraformaldehyde and subjected to analysis of the optical density with confocal laser scanning microscopy (Leica, Wetzlar, Germany).

**FRAP.** Cultured cells were loaded to 70–80% with 10 μM of the fluorescent dye 6-carboxyfluorescein diacetate containing Ca²⁺ and Mg²⁺ at 37°C and 5% CO₂, and samples were rinsed three times with PBS (15). FRAP was measured using the confocal laser scanning microscope (Leica). The dye was excited at 488 nm with 500-mW laser power, and its emission was recorded at 570 nm. A density filter was used to minimize photobleaching at 513 nm. The mean fluores-

Fig. 4. Western blot analysis of the expression of Cx43 protein in cultured detrusor cells (left). The optical density (OD) rate (normalized by inner control β-actin) for comparison between control and DO groups is plotted in the histogram (right). Values are means ± SE; n = 6. **P < 0.01.

Fig. 5. Scrape loading dye transfer (SLDT) analysis of the coupling between cultured detrusor cells. A: Lucifer yellow entered the cells at the border of the scratch and reached cells as far as 90 μm away from the scratch in the control group and 400 μm in the DO group. Red arrow indicates scrape; yellow arrow indicates the distance of dye transfer. Magnification, 200. B: average fluorescence intensity of the scrape-loaded area in DO (n = 29) and control groups (n = 24). **P < 0.01.
cence intensity at different scannings was recorded. The cells were exposed to 10 scans with an interval of 30 s, and the fluorescence recovery rates were evaluated over a period of 4 min after bleaching. According to the Leica TCS NT instructions, all the manipulations were completed within 30–40 min. The decrease of fluorescence in single cells with no GJ contact with other cells was measured as a control to normalize the bleaching caused by the excitation light. The mean normalized fluorescence recovery rate was calculated from the formula $K = (I_b - I_b^0)/(I_u - I_u^0) \times 100\%$, where $K$ is the mean fluorescence recovery rate, $I_b$ is the fluorescence intensity of the bleached area after fluorescence recovery, $I_b^0$ is the fluorescence intensity of the bleached area immediately after the bleaching, $I_u$ is the fluorescence intensity of the unbleached area, and $I_u^0$ is the background fluorescence. 18β-Glycyrrhetinic acid (18β-GA), a GJ blocker, was used to inhibit cell-cell communications in the cells of the DO group and to evaluate the inhibitory efficacy on the increased bladder excitability induced by the changes in intercellular communication.

Statistical analysis. All data are means ± SE. The original data from SLDT and FRAP were acquired with a TCS NT microscope and analyzed using Microsoft Excel. A nonparametric Mann-Whitney test was used for the RT-PCR data, and other data were tested using a $t$-test (SPSS 11.0 software). $P < 0.05$ was considered statistically significant.

RESULTS

General evaluation. The bladder size increased dramatically in rats 6 wk after PBOO compared with the controls. The bladder wet weights were 120.0 ± 6.45 mg in control rats ($n = 13$) and 630.8 ± 71.25 mg in the DO group ($n = 23$, $P < 0.001$). Other urodynamic parameters were compared, and the results show that residual urine, bladder capacity, and pressure in the DO group were higher than in the control group, whereas bladder compliance was decreased in DO rats compared with control rats. All differences are statistically significant (Table 2).

At the light microscopy levels, the detrusor cells in the control group were regularly arranged and of a fusiform shape,
whereas in the DO group the cells were arranged irregularly and exhibited hypertrophy and multiple shapes (Fig. 1). At the electron microscope level, irregularly arranged myofilaments were displayed in DO cells. The quantity of intermediate junctions in control and DO groups was 13.3 ± 3.9 and 65 ± 1.2 per 10HP (high-power field; n = 23), and the numbers of GJs was 4.7 ± 1.1 and 16.3 ± 3.6 per 10HP (n = 13), respectively (Fig. 2).

Expression of Cx40, Cx43, and Cx45. RT-PCR analysis showed that Cx40, Cx43, and Cx45 all were expressed in detrusor myocytes of both control and DO groups. The brightness of the bands reverse-transcribed from the mRNA of Cx40 and Cx43 electrophoresed on the agarose gel is shown in Fig. 3. Semiquantitative analysis showed that the brightness of the bands of Cx43 and Cx40 mRNA in the DO myocytes was 5.4- and 2.9-fold stronger than in control myocytes, but there was no significant difference in Cx45 mRNA between the two groups. Western blot analysis revealed that Cx43 protein levels were markedly increased after 6 wk of outflow obstruction, about threefold higher than the initial control values (Fig. 4). Taking into account the approximately fivefold increase in bladder weight, the absolute amounts of Cx40, Cx43, and Cx45 mRNA and Cx43 protein in the detrusor increased ~15-, 27-, 5-, and 15-fold, respectively, in the DO group.

SLDT. The techniques of intercellular transfer of hydrophilic dyes after scrape loading and photobleaching worked well in detecting the changes in function of the gap junctions (5, 10). The distance over which Lucifer yellow spread through the cells from the border of the scratch in the DO group (n = 35) was significantly further than in the control group (n = 28). A typical example is shown in Fig. 5A, where the distance of spread was 400 μm from the scratch in the DO group but only 90 μm in the control group. The fluorescence intensity of the scrape-loaded area in the DO group also increased dramatically. The mean value of the area in the DO group (n = 29)

Fig. 7. FRAP in DO detrusor cells. A: FRAP at different time points. The scale bar in A applies to all the micrographs. Red arrow indicates a bleached cell; yellow arrow indicates a single cell. B: changes of fluorescence intensity of 3 different kinds of cells cultured from overactive bladders.
increased 5.6-fold compared with the matched controls ($n = 24$), as shown in Fig. 5B.

**FRAP.** FRAP was detected in the control and DO bleached cells but not in single bleached cells. Representative examples of micrographs of cells from both control and DO groups before and 2 and 4 min after bleaching are shown in Figs. 6A and Figs. 7A, respectively. The fluorescence intensity of bleached cells in both groups had declined immediately after bleaching and then gradually recovered and reached a maximum 4 min after the bleaching. However, the fluorescence recovery rate in the cells of the DO group was much higher than in the cells of the control group. The mean fluorescence recovery rate after 4 min was 80.5 ± 8.3% for the DO group and 55.3 ± 6.4% for the control group ($n = 6$, $P < 0.01$; Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>0 min</th>
<th>1 min</th>
<th>2 min</th>
<th>3 min</th>
<th>4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>221.129±12.05</td>
<td>105.51±8.36</td>
<td>110.816±9.32</td>
<td>113.667±7.64</td>
<td>121.862±8.27</td>
<td>122.387±10.51</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$.

Figures 6B and 7B show the fluorescence intensity of bleached cells, unbleached cells and single cells in the control ($n = 38$) and DO groups ($n = 61$), respectively, plotted against the time after the bleaching. Recovery only occurred in bleached cells, and the recovery rate in DO cells was faster than in control cells. Another study to evaluate the difference of fluorescence intensity in the control and DO groups at different time points is shown in Fig. 8A, and the total recovery of the bleached cells in both groups is shown in Fig. 8B. Measurements of the absolute intensity show that in the unbleached cells, the fluorescence intensity decreased with time, in contrast to the intensity in the bleached cells, particularly in cells from the overactive detrusor, which recovered to some extent. Administration of the GJ blocker 18β-GA caused a concentration- and time-dependent inhibition of fluorescence recovery. 18β-GA in concentrations of 10, 20, 40, 80, and 160 μM suppressed the rate of FRAP to 47.6, 30.2, 16.7, 14.7, and 7.5% 4 min after bleaching compared with the control (Fig. 9).

**DISCUSSION**

The results from our rat model 6 wk after PBOO are all consistent with other studies (20). Residual urine, bladder capacity, bladder wet weight, and micturition threshold volume and pressure were increased, whereas bladder compliance was decreased. For further studies in vitro, only the rats with DO after PBOO were chosen for comparison with the sham-operated rats. This has produced a reliable model.

Rational treatment of patients with DO secondary to outlet obstruction has been hampered, because the pathogenesis of DO is not completely understood. To date, different neural receptor antagonists and agonists commonly have been used with a rationale mainly based on a supposed neurogenic etiol-
ogy, but the outcomes are inconsistent or the treatments generate intolerable side effects (19, 12). It seems likely that obstructive DO involves a much more complex pathogenesis than initially thought, and successful therapy may require that several integrated factors are taken into account, e.g., altering in myosin isoform expression in bladder myocytes (2) and changes in cell-cell contact (4, 6), among others. In the present study, we have demonstrated that the Cx43 and cell-cell communication in GJs of DO cells was increased significantly compared with the control, supporting the notion that myogenic changes may play an important role in the development of DO.

GJs, a special structure in cell membranes, form aqueous channels interconnecting the cytoplasm of adjacent cells for cytoplasmic exchange without involving extracellular elements. GJs bridge the intervening extracellular space by dock- ing two hemichannels of each adjacent cell. Each hemichannel is an oligomer consisting of six connexin molecules, which make up GJ plaques (17). On the basis of this knowledge, one can expect that the more GJ plaques, the more the cellular connectivity and the higher the excitability of the detrusor. GJs allow the passage of small molecules, ions, and metabolites whose molecular weight is <1 kDa through their low-resistance intercellular pores, which favors direct intercellular electrical and metabolic coupling (11, 27). Our findings of the increase of GJs and enhanced connectivity could be regarded as one of the mechanisms in the pathogenesis of DO.

Biophysical and electrophysiological characteristics of the junctional recordings on the short-term cultured human detrusor myocytes show that Cx43 is a major functional connexin protein present in normal human bladder smooth. There is clear evidence for GJ coupling between human detrusor myocytes and, moreover, for a potentially important role for altered intercellular communication in the pathogenesis of bladder dysfunctions (25). An increase in Cx43 transcription levels correlates with the time after PBOO with a maximum level at 6 wk (6). In agreement with these findings, we showed by PCR and Western blot analysis that Cx40, Cx43, and Cx45 were much elevated (5- to 27-fold compared with the control) in the cells 6 wk after PBOO, indicating that these connexins are essential for cell-cell communications in detrusor myocytes.

Presently, approaches for evaluating the connexin-mediated cellular connectivity mainly depend on molecular and biophysical techniques, such as SLDT, microinjection and dye transfer assay (MDTA), and FRAP, among others, because of the apparent inability to visualize morphologically definable junctional plaques at the levels of light and electron microscopy in normal bladders. However, it is valuable to determine the morphological changes of GJs in DO detrusor. In this study we used light and electron microscopy and found that the cells in the DO group had multiple shapes and were irregularly arranged, with a decrease of intermediate junctions and an increase of GJs, clearly different from the cells of the control group.

With RT-PCR evaluation, Cx40, Cx43, and Cx45 mRNA in the DO group were increased ~15-, 27-, and 5-fold compared with the control, and Western blot analysis showed an increase of ~15-fold. Cellular connectivity by FRAP and SLDT studies revealed that the function of the gap junctions was also increased in the DO group. All these changes suggest that GJ channels in the DO group allow the passage of small mole- cules, ions, and metabolites to bring about the depolarization of adjacent cell membranes and provide an extensive intercellular electrical communication, which promotes the electrical activity of local cells to spread to the entire bladder and results in unified nonvoiding contractions. Therefore, it is reasonable to speculate that this pathological process in cellular connectivity tends to increase the bladder excitability and eventually leads to the development of DO. This assumption may offer a rational approach for treating patients with DO following PBOO by using GJ blockers or antagonists to suppress the junctional pathway.

18β-GA is a GJ blocker for electrical coupling. Hashitani et al. (14) showed an inhibitory effect of 18β-GA (40 μM) on the cell-to-cell fluorescence conduction in the detrusor cells in guinea pig. The report of Guan et al. (13) also revealed that the signal transmission between the liver endothelial cells was blocked by 18β-GA. The observation of Santicioli et al. (24) on pelvireteral junctional electrical coupling showed that 18β-GA (30 μM) can inhibit intercellular electrical coupling. In agreement with these reports, we have shown that 18β-GA caused a concentration- and time-dependent inhibition of the cellular connectivity in rat DO cells. The effect could be caused by 18β-GA disassembling and/or dephosphorylating Cx43. The potential feasibility of the treatment of DO with 18β-GA agonist is suggested by this study, and this feasibility needed to be further studied with control detrusor, and this is our ongoing project with systematic and concentration-dependent evaluation.

Conclusions. The quantity of GJs and expression of Cx40, Cx43, Cx45 mRNA and Cx43 protein were increased in DO cells, and the cellular connectivity increased as shown by SLDT and FRAP techniques in DO cells after 6 wk of PBOO. The findings provide a theoretical rationale for clinical use of GJ blockers in the treatment of patients with DO induced by PBOO.

ACKNOWLEDGMENTS

We are grateful to Prof. Alison Brading for revising this manuscript.

GRANTS

The present study was supported by the National Natural Science Foundation of China (30500505, 30271304).

REFERENCES


