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*<Original Article>*

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**Running title.** Effect of PDE3i treatment on female rat obstructed bladder.

**Keywords.** Cilostazol, PDE3 inhibitor, bladder outlet obstruction, rat

**Conflict of interest.** None declared.

**Abstract**

**Objectives** To investigate whether bladder dysfunction after bladder outlet obstruction (BOO) could be altered by treatment with cilostazol, a phosphodiesterase 3 inhibitor (PDE3i).

**Methods** Twelve-week-old female Sprague-Dawley rats were divided into five groups; group 1 and 2, sham operated rats (each 4 rats); group 3-5, BOO rats (each 6 rats). Group 1 and 3 rats were given normal diet, group 2 and 5 rats were given high dose PDE3i diet, and group 4 rats were given low dose PDE3i diet. PDE3i was given within diet from the day of surgery. Four weeks after BOO, the bladder was excised and dissected into four longitudinal strips for isometric organ-bath assay. Contractile responses of bladder strips to electrical field stimulation (EFS), carbachol and KCl was determined for each group.

**Results** BOO induced a significant increase in bladder weight in group 3-5 compared with group 1 and 2. PDE3i treatment did not affect bladder weight in either sham or BOO rats. Contractile forces in response to EFS, carbachol and KCl in group 3 were about 20-40% of those in group 1. Contractile responses to EFS or KCl in PDE3i treated BOO rats were not significantly different from those in normal diet treated BOO rats. Only high dose of PDE3i treatment in BOO rats caused a statistically significant increase in the response to carbachol compared with normal diet treated BOO rats.

**Conclusions** PDE3i has a small but significant protective effect on the contractile

dysfunction induced by 4-weeks BOO in rats, although the increase in bladder mass was not altered. PDE3i could be a useful protection against contractile dysfunction of the obstructed bladder.

**Introduction**

LUTS are highly prevalent among elderly men and women, and have a significant impact on the patients' quality of life. The cause of LUTS is multifactorial and includes BOO, bladder ischemia, autonomic sympathetic overactivity, and so on [1]. Increasing evidence has shown that ischemia and reperfusion are major etiologic factors in the progression of bladder dysfunction induced by BOO, and that part of the damage is due to the generation of free radicals and the resultant cellular and subcellular membrane peroxidation [1]. The importance of ischemia as an etiologic factor in bladder dysfunction has been supported by recent animal studies in which bladder ischemia was experimentally created by BOO, bladder overdistension and atherosclerosis [2-7]. The evidence has suggested that bladder ischemia causes significant bladder dysfunction and leads to decreased contractile responses of the bladder.

$\alpha_1$ - blocker has been shown to have a protective effect on bladder function of the rat after BOO or bladder overdistension possibly through an improvement of bladder ischemia [6,7]. In clinical practice, $\alpha_1$ - blocker is widely used as a first-line treatment for male patients with LUTS that are associated with BOO due to BPH (LUTS/BPH). $\alpha_1$ - blocker improves chronic ischemia of the lower urinary tract in patients with LUTS/BPH [8]. In addition to $\alpha_1$ - blocker, PDE5i has also been shown to have a protective effect on bladder function of the rat after BOO [9] and to increase contractile force of the bladder in normal rats [10]. These basic studies are translated into clinical urology and PDE5i is

used for treating male LUTS [11-13].

Cilostazol (6[4-(l-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3, 4-dihydro-2(1H)-quinolinone), a quinolinole derivative acting as a PDE3i, has been clinically used since 1985 in Japan and since 1996 in the United States as an antiplatelet/antithrombotic agent for patients with arterial occlusive diseases. Cilostazol exerts a protective effect on various vascular tissues following acute ischemia [14-16], and it alleviates the symptoms of intermittent claudication in patients with peripheral vascular disease. This compound is a selective PDE3i, and it raises intracellular cyclic AMP content and activates PKA. These events result in antiplatelet aggregation and peripheral vasodilation [17]. The role of the NO pathway in the pharmacological action of cilostazol remains largely unknown.

We hypothesized that PDE3i might have a potential for increasing bladder blood flow and improve bladder dysfunction caused by BOO. The present study was conducted to investigate whether bladder dysfunction after BOO could be altered by treatment with a PDE3i.

## Materials and Methods

### *Animals*

Twelve-week-old female Sprague Dawley rats (CLEA, Tokyo, Japan) were used. The rats were housed in a room controlled at 21-25 ℃ and 45-65 % humidity for at least

one week prior to the experiments. Food and water were supplied freely. All experimental procedures were approved by Asahikawa Medical University Institutional Animal Care and Use Committee.

***BOO model***

The bladder outlet was partially obstructed by the methods described previously [9]. Briefly, rats were anesthetized with diethyl ether and pentobarbital sodium, and then placed in a supine position. After sterilization with an iodine/alcohol mixture, the abdominal cavity was opened by midline incision to expose the urethrovesical junction. Proximal urethra was loosely tied with a 19 G needle using 2-0 silk thread and the needle was removed to produce partial BOO. Incision was closed and Penicillin G potassium (0.1 mg/kg) was given i.m. The same operation was performed in sham-operated rats without tying the thread. Rats were divided into five groups; group 1 and 2, sham operated rats (each 4 rats); group 3-5, BOO rats (each 6 rats). Group 1 and 3 rats were given normal diet, group 2 and 5 rats were given high dose PDE3i diet, and group 4 rats were given low dose PDE3i diet.

***Drugs***

Cilostazol was a kind gift from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Cilostazol was mixed with food and given from the day of surgery. In the present study, we examined a low dose (0.003% in feed) and a high dose (0.01% in feed) of cilostazol on the basis of prior reports, in which oral cilostazol administration to rats resulted in different

blood concentrations between male and females rats with several ten-fold higher levels in female rats compared to male rats, and the doses of 0.03%, 0.1% and 0.3% were used in oral cilostazol administration to male rats [18-21].

***Contractility Measurements in Isolated Bladder Smooth-Muscle Strips***

Contractile responses were measured as described previously [9,10]. The bladder was excised, weighed, and full-thickness longitudinal strips of about 3 × 10 mm were taken from the bladder body. A part of the remaining bladder tissue was fixed overnight in 10% formaldehyde solution, then embedded in paraffin for histological studies. The strips were mounted in isolated muscle baths containing Tyrode's solution and equilibrated for 1 hr. Bladder strips were stimulated with electrical field stimulation (EFS; 2, 8, 32 Hz), carbachol (20 μM), and KCl (120 mM). Responses were recorded isometrically, and maximal tensions were compared.

***Histological examination***

Formalin fixed tissue strips were dehydrated, embedded in paraffin and sectioned every 5μ for hematoxylin-eosin staining, and azan-mallory staining to identify smooth muscle (red) and connective tissue (blue).

***Statistical Analysis***

All values were expressed as the mean ± SEM. One way ANOVA was used to analyze statistical significance. Differences were considered to be significant at a P-value $< 0.05$.

**Results**

Adverse effects by PDE3i medication, such as a bleeding tendency, were not observed in this study. The effects of cilostazol on body weight and bladder weight are shown in **Figure 1**. Neither BOO surgery nor cilostazol treatment had any effects on body weight. BOO induced a significant increase in bladder weight by 4-6 folds (group 3 vs group 1). Bladder weights of cilostazol treated BOO rats (groups 4 and 5) were not significantly different from those of normal diet treated BOO rats (group 3).

Mean contractile response to EFS in BOO rats (group 3) was 21.2 %, 21.3 % and 25.5 % at 2, 8, and 32 Hz, respectively, of those in normal diet sham control rats (group 1). Contractile responses to EFS in cilostazol-treated BOO rats were not significantly different from those in normal diet treated BOO rats **(Figure 2)**. Contractile responses to carbachol and KCl in BOO rats (group 3) were 40.3 % and 39.6 % of those in normal diet sham control rats (group 1), respectively. Contractile responses to KCl in cilostazol-treated BOO rats were not significantly different from those in normal diet treated BOO rats. Contractile responses to carbachol in cilostazol-treated BOO rats showed a dose-dependent increase and only the high dose cilostazol diet caused a statistically significant increase in the response compared with normal diet treated BOO rats.

In BOO rats (group 3), there was a marked increase in hypertrophied smooth muscle cells and spaces between smooth muscle bundles were evident **(Figure 3)**.

Cilostazol treatment had no significant effects on histological profiles **(Figure 3)**.

## Discussion

Bladder ischemia has been shown to be one of pathophysiological factors of LUTS/BPH. Bladder ischemia is caused by reduced bladder blood flow associated with aging, arteriosclerosis and BOO. Recent reports indicated a strong relationship of LUTS/BPH with metabolic syndrome and erectile dysfunction (ED) [22,23], and many studies demonstrated that PDE5i, agents used for the treatment of ED, also improved LUTS in patients with BPH [11-13]. The results of these studies implicate that LUTS/BPH are partially attributed to disturbance in the NO/cGMP pathway caused by reduced bladder blood flow, and that PDE5i could improve LUTS/BPH by activating the NO/cGMP pathway. PDEs convert cAMP and cGMP to inactive 5'-AMP and 5'-GMP, and influence downstream intracellular signaling. PDEs consist of 11 broad families (PDE1–PDE11), and their distribution and function differ according to species [24]. In addition, PDE family is known to regulate the concentration by balancing the enzyme activity and to play an important role in signal transduction. PDE isoforms that hydrolyse cAMP include PDE1, PDE2, PDE3, PDE4, PDE5, PDE7, PDE8, PDE10 and PDE11. Other than PDE5, PDE4 has been target for basic research of bladder function. Snyder et al. showed the involvement of PDE4 in the control of bladder smooth muscle tone in vitro [25]. A selective PDE4i effectively suppressed detrusor overactivity in rats with BOO [26]. Based on accumulating

evidence, PDE inhibitors have been suggested to have a potential for therapeutic agents to treat bladder dysfunction in LUTS/BPH [27]. Particularly PDE inhibitors are likely to protect against bladder dysfunction by increasing bladder blood flow.

Among PDE family members, PDE3 is specifically present in the platelet, cardiac muscle and vascular smooth muscle, and is involved in the regulation of intracellular cGMP and cAMP levels and the control of cardiovascular system [28]. PDE3i are effective in improving cardiomyopathy, pulmonary hypertension and related conditions, and have effects of suppressing platelet aggregation and enhancing lipolysis. Thus it seems likely that PDE3i improves bladder blood flow. Direct effect of PDE3i on bladder smooth muscle is also likely. In recent years, PDE3i are widely used for prevention and treatment of cerebral infarction and myocardial infarction in clinical practice. The results of the present study suggest that PDE3i provides potential protection against deterioration of bladder function in patients who take PDE3i possibly through the improvement of bladder blood flow. Furthermore, if PDE3i has a potency to improve bladder function, it will also provide scientific evidence for a new treatment approach for LUTS/BPH in which PDE3i can be used to prevent and manage contractile dysfunction of obstructed bladder in a similar fashion to PDE5i [9] and aspirin [29]. No other studies from such viewpoints have been reported to date, and this study could explore a new indication of antiplatelet agents including PDE3i in treatment strategy for LUTS/BPH.

In the present study, actual bladder blood flow was not measured. However, it

seems that PDE3i improves reduced bladder blood flow in BOO by their antiplatelet and vasodilating actions and, as a result, suppresses deterioration of bladder function (detrusor contraction) in a similar mechanism to aspirin [29]. The effect of PDE3i was nearly the same to that of PDE5i in a previous report [9], indicating a potential use of PDE family in protecting bladder function through selective use.

**Conclusions**

Cilostazol, a PDE3 inhibitor, has a modest but significant protective effect on contractile dysfunction induced by BOO in rats, although the increase in bladder weight was not altered. Bladders of the same weight showed significantly better responses to carbachol after treatment with cilostazol. Thus cilostazol may provide protection against contractile dysfunction of the bladder in patients with LUTS/BPH.

**Abbreviations & Acronyms**

PDE3i = phosphodiesterase 3 inhibitor

AMP = adenosine monophosphate

PDE4i = phosphodiesterase 4 inhibitor

PDE5i = phosphodiesterase 5 inhibitor

BOO = bladder outlet obstruction

BPH = benign prostatic hyperplasia

LUTS = lower urinary tract symptoms

ED = erectile dysfunction

EFS = electrical field stimulation

KCl = potassium chloride

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**Figures Legends**

**Figure 1.** Effects of cilostazol on body weight and bladder weight. * p<0.05 vs group 1 (sham-operated rats given normal diet)

**Figure 2.** Effects of cilostazol on electrical field stimulation (EFS)-, carbachol- and KCl-induced contraction of rat bladder strips. *; p<0.05 vs group 1 (sham-operated rats given normal diet), **; p<0.05 vs group 3 (BOO rats given normal diet)

**Figure 3.** Effects of chronic treatment of cilostazol on bladder histology. Rat bladder was stained with hematoxylin-eosin (HE) and azan-mallory (Azan). Representative pictures were shown.

**Figure 1**

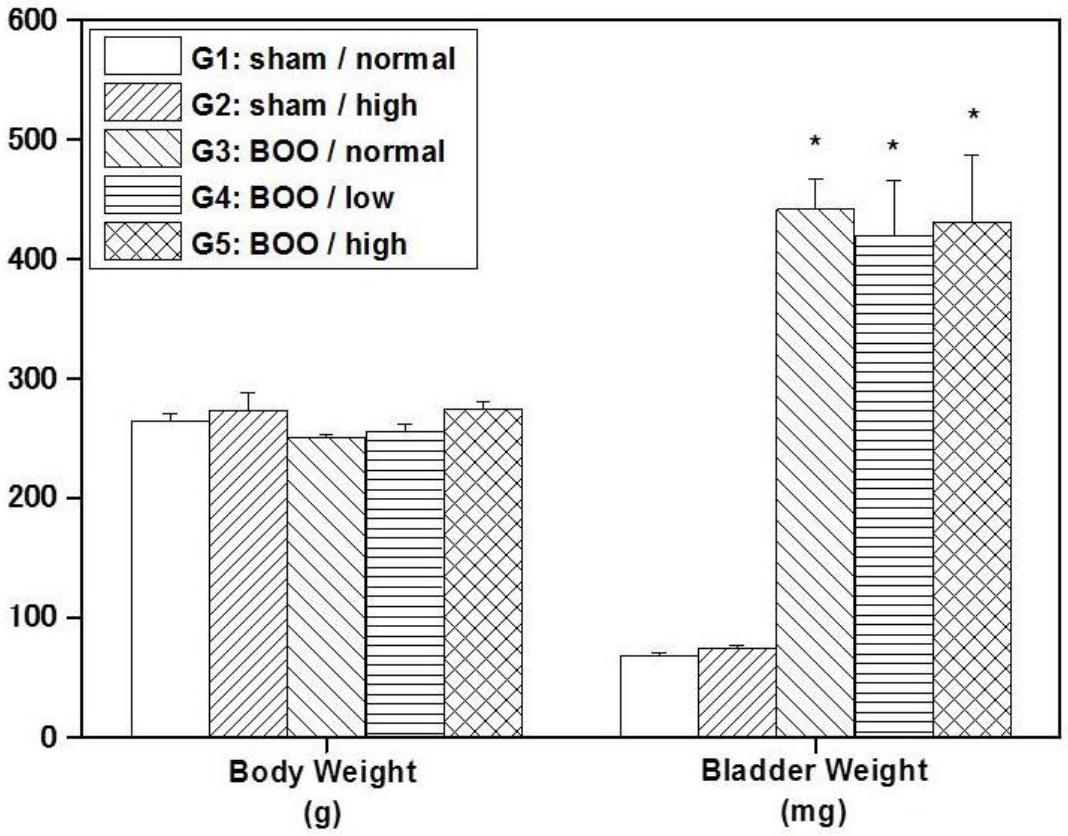

600
500
400
300
200
100
0
G1: sham / normal
G2: sham / high
G3: BOO / normal
G4: BOO / low
G5: BOO / high
*
*
*
Body Weight
(g)
Bladder Weight
(mg)

**Figure 2**

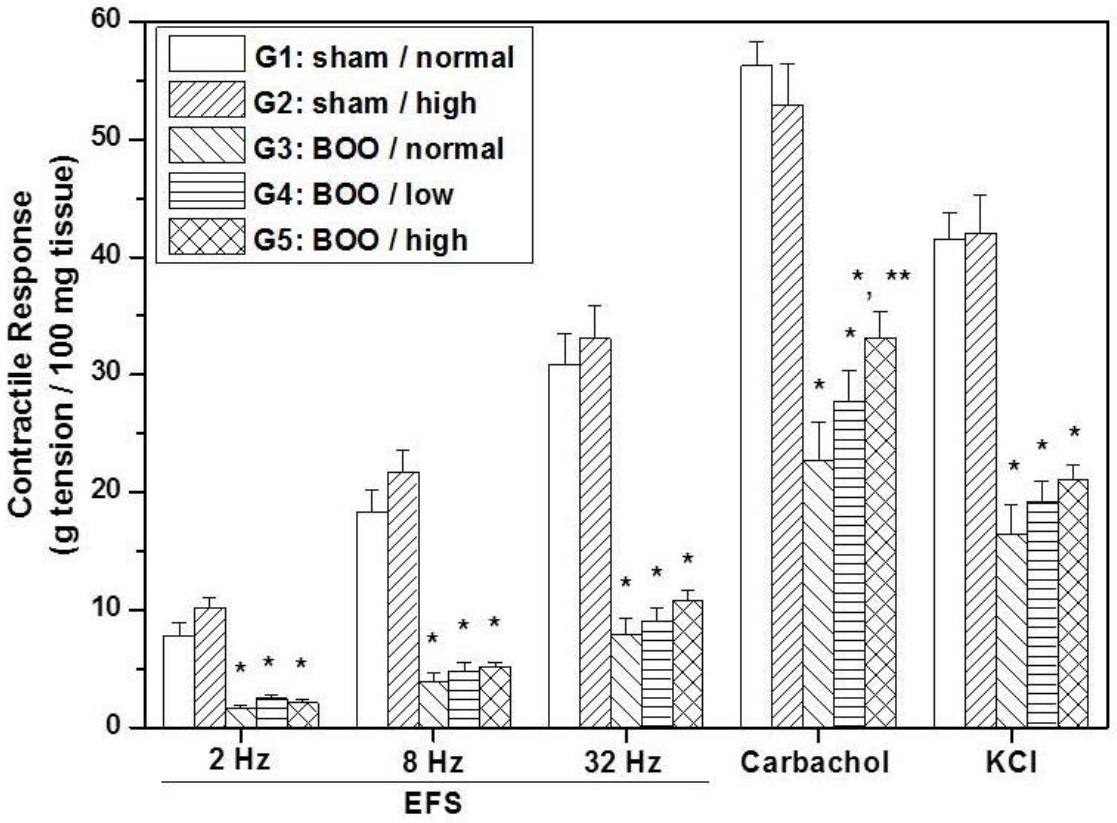
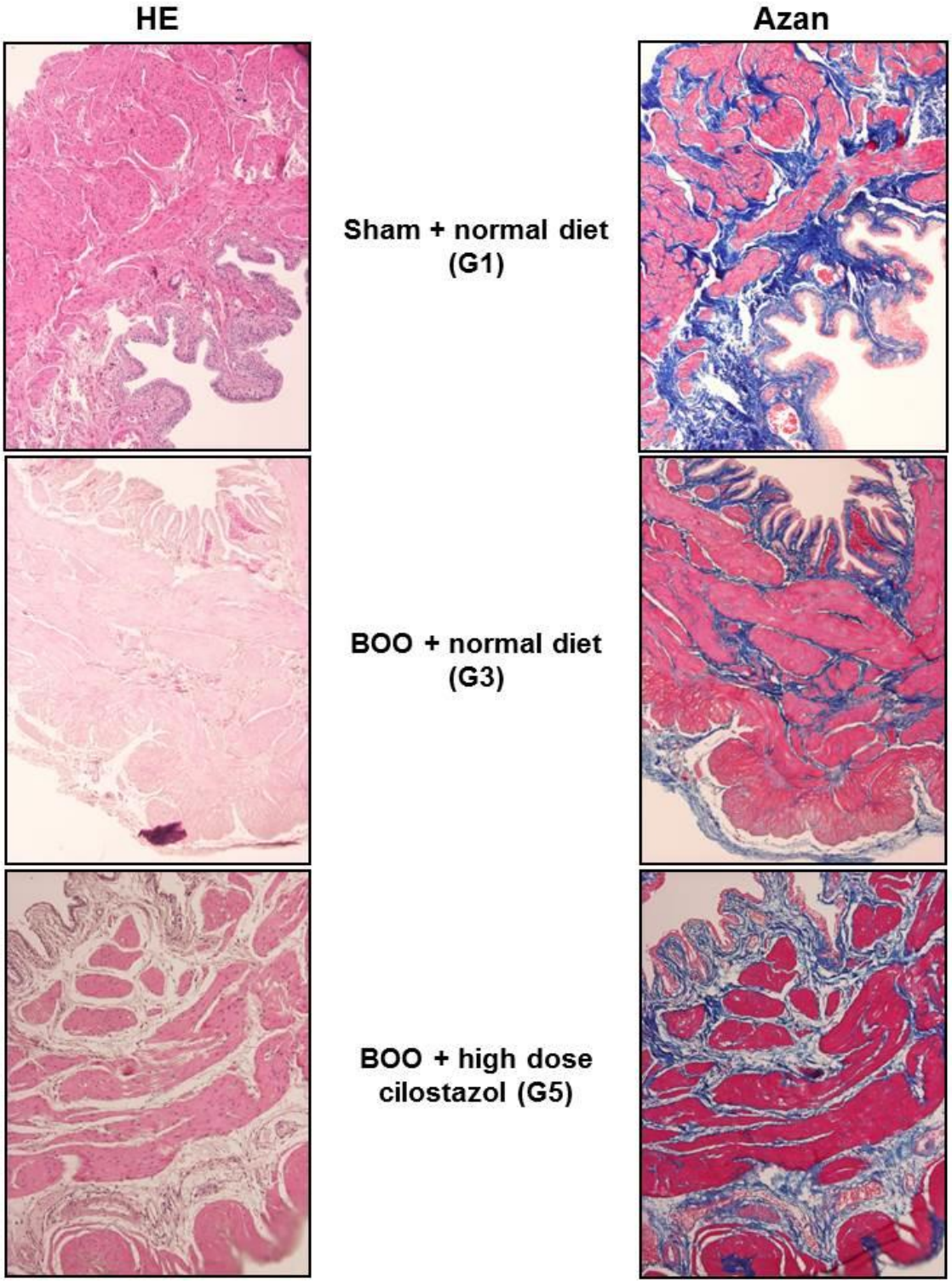

G1: sham / normal
G2: sham / high
G3: BOO / normal
G4: BOO / low
G5: BOO / high
Contractile Response
(g tension / 100 mg tissue)
60
50
40
30
20
10
0
2 Hz
8 Hz
32 Hz
EFS
Carbachol
KCl

**Figure 3**


HE
Azan
Sham + normal diet (G1)
BOO + normal diet (G3)
BOO + high dose cilostazol (G5)