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Kokubun S[1], Aikawa K[1], Shishido K[1], Ishibashi K[1], Yanagida T[1], Nomiya M[1], Takahashi N[1], Shiomi H[1], Ogawa S[1], Kawashima Y[1], Yamaguchi O[1], Hayashi Y[2]

1. *Fukushima Medical University,* ***2.*** *Taiho Pharmaceutical Co.,Ltd. Drug Discovery Research Center*

PROTECTION OF NEURONS FROM DENERVATION PREVENTS BLADDER DYSFUNCTION FOLLOWING BLADDER OUTLET OBSTRUCTION IN RAT

Hypothesis / aims of study

Detrusor dysfunction associated with bladder outlet obstruction (BOO) has been long recognized. There is much evidence indicating that outlet obstruction causes denervation in bladder smooth muscle. The pharmacological evidence of denervation is also drawn from the findings that detrusor muscle from the obstructed bladder exhibits supersensitivity to acetylcholine (main excitatory neurotransmitter) and reduction in nerve-mediated contraction.

These changes are thought to be the basis of the abnormal behaviour of obstructed bladder,

i.e., detrusor overactivity coexisting with the decreased contractility. Thus denervation may underlie the development of detrusor dysfunction. If denervation of the detrusor muscle can be prevented, it would be a novel treatment modality for detrusor dysfunction secondary to obstruction. Recently, a new compound (TAC-302), cyclohexenone long-chain alcohol derivative, has been shown to possess some neurotrophic activity in pathologic conditions such as brain infarction. Therefore, using TAC-302, we investigated whether denervation supersensitivity and the decreased contractility would be improved by protecting neurons against denervation. In addition, the effects of this TAC-302 on the micturition behaviour of rats with BOO were also evaluated.

Study design, materials and methods

At 9 weeks of age 15 male Spraque-Dawley rats received partial BOO (n=10) or sham surgery (SHAM group; n=5) were used. After surgery, 5 BOO rats received daily administration of 8 mg/kg TAC-302 intraperitoneally for 4 weeks, while the other 10 rats had no treatment. Four weeks following surgery, micturition behaviour was observed in metabolic cage. Then all rats were sacrificed and bladder was taken for morphological and pharmacological studies. Full thickness bladder serial frozen sections were made for PGP9.5 (a pan neuronal marker) immunohistochemical study. In addition, muscle strips from bladder dome were suspended in a 25 ml organ bath containing Krebs' solution. A dose response curve to carbachol (CCh) was produced by adding increasing concentrations of the drug. Transmural nerve electrical stimulation was performed to evaluate contractility of the detrusor muscle.

Results

Compared to SHAM group, bladder weight increased significantly following obstruction. Neurotrophic compound (TAC-302) had no effect on increased bladder weight induced by obstruction. The metabolic cage study showed that BOO caused a significant increase in voiding frequency and a significant decrease in the mean voided volume, while TAC-302 increased mean voided volume significantly (Table 1). Immunohistochemical study showed that PGP9.5 positive nerve fibers were decreased in obstructed bladders, and TAC-302 well preserved these PGP9.5 positive nerves (Fig. 1).

The CCh dose response curves demonstrated a significant leftward shift for bladder muscle from BOO rats, suggesting that detrusor muscle were supersensitive to exogenous CCh (Fig. 2). In addition, muscle strips from BOO rats showed a significant reduction in nerve-mediated contractile response (Fig. 3). TAC-302 significantly reduced supersensitivity and preserved decreased nerve-mediated detrusor contraction (Fig. 2 and 3).

Interpretation of results

BOO causes denervation of bladder smooth muscle that results in supersensitivity to CCh and reduction in nerve-mediated contraction. The results of voiding behaviour suggest that BOO impairs storage function. TAC-302 may improve these changes associated with BOO by preventing the development of denervation.

Concluding message

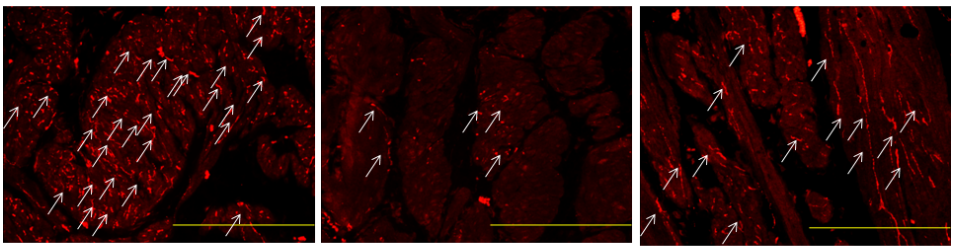
Clinical implication from this study is that using a neurotrophic agent such as TAC-302, neurons will be protected against denervation, which may have beneficial effects in the treatment of detrusor dysfunction secondary to obstruction.

Table 1

	SHAM	BOO	BOO + TAC-302
24-hr water consumption (ml)	32.4 ± 3.6	31.1 ± 2.7	32.3 ± 2.7
24-hr voided volume (ml)	16.3 ± 1.6	22.4 ± 2.8	23.6 ± 2.3
24-hr frequency	14.7 ± 2.1	45.3 ± 9.0 *	30.9 ± 2.3
Mean voided volume (ml)	1.15 ± 0.08	0.53 ± 0.04 *	0.77 ± 0.03 * †

Each value represents the mean ± SEM.
* P<0.05, significant difference from SHAM † P<0.05, significant difference from BOO

Fig. 1



A representative immunostaining for PGP9.5 positive nerve fibers from bladder muscle of SHAM, BOO and BOO + TAC-302 (Bars 10 µm)

Fig. 2

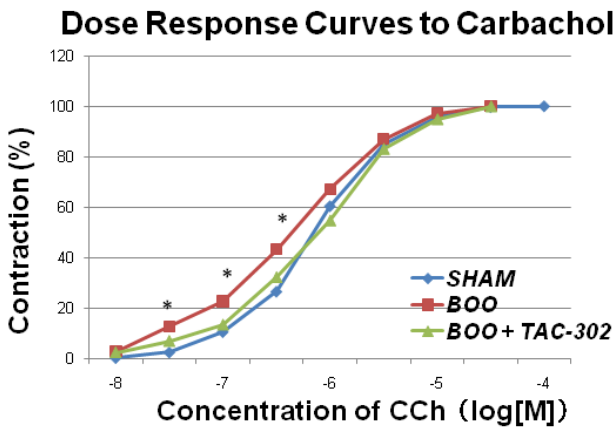
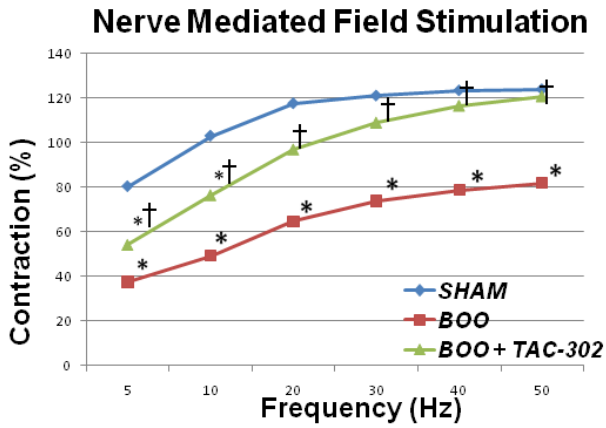


Fig. 3



* p<0.05, significant difference from SHAM
† p<0.05, significant difference from BOO

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Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL

Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Fukushima Medical University Ethical Committee**