Effects of combination drug therapy against OAB on micturition parameters and release of nitric oxide in the healthy and inflamed rat bladder

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Introduction and Aims

Overactive bladder (OAB) is a diagnosis which encompasses bladder hyperactivity and hypersensitivity and affects a large number of patients worldwide. The symptoms experienced by patients vary, especially when comparing OAB patients with an otherwise healthy bladder to those with concomitant inflammation. Further, it is well-known that the most common treatment against OAB, namely antimuscarinics, is less effective in patients with concomitant cystitis. Not long ago, mirabegron, a selective β3-adrenoceptor agonist, was approved for the treatment of OAB and studies have indicated that one benefit of mirabegron is its efficacy also during cystitis. While the most common treatment against OAB worldwide is still monotherapy, a combination therapy including an antimuscarinic and mirabegron has been suggested as the new golden standard treatment. While a direct effect on the detrusor is assumed, some reports on mirabegron have suggested that part of its relaxatory effect is exerted via release of nitric oxide (NO).

The aim of the current study was to compare how micturition patterns are affected by combination drug therapy against OAB in health and cystitis and if the efficacy of the drugs can be linked to altered release of NO.

Methods

A total of 32 adult male Sprague-Dawley rats were used in the current study, which followed national guidelines for the care and use of laboratory animals and was approved by the local ethics committee (permit #196/2013). Each rat was pre-treated twice daily with either saline (1 mL/kg s.c.) or a combination drug treatment (B, D; mirabegron 0.6 mg/kg/day s.c. and tolterodine 0.05 mg/kg/day s.c.). Both combination drug treatment and cystitis caused a significant increase in the levels of NO. Mesmeric antagonism most likely inhibits release of nitric oxide, if anything.1 This is highly interesting in the context of understanding the mechanisms underlying the beneficial effect of mirabegron. However, it should be noted that inflammation per se increases the levels of NO and the level of NO is not significantly different between saline pre-treated and drug pre-treated rats. This indicates that inflammation increases the release of NO, if anything.1

Results

In control rats, the volume/micturition was not affected by the combination drug therapy (1.03±0.23 mL and 0.95±0.11 mL in saline pre-treated and drug pre-treated controls, respectively; Fig 2A). Likewise, no difference could be seen when comparing number of micturitions per hour (1.03±0.23 and 0.94±0.19 in saline pre-treated and drug pre-treated controls, respectively; Fig 2B). However, induction of cystitis with CYP caused a non-significant decrease in volume/micturition (from 1.03±0.23 to 0.74±0.06 mL; Fig 2A) and a significant increase in the number of micturitions per hour (from 1.03±0.23 to 1.85±0.18 mict/h; Fig 2B). When pre-treating the CYP-treated (indflamed) rats with the combination of tolterodine and mirabegron, this micturition pattern was normalized and no significant differences as compared to saline pre-treated controls were seen (1.21±0.12 mL vol/mict and 1.07±0.09 mict/h, respectively; Fig 2). Similarly, the micturition parameters in the inflamed rats which were pre-treated with the combination of drugs was significantly different as compared to saline pre-treated inflamed rats (Fig 2). Release of NO, measured by volumetry as amount of nitrite in urine, increased significantly upon treatment with the drug combination, both in controls and rats with CYP-induced cystitis (from 25.86±1.96 in saline pre-treated controls to 42.99±5.51 µM in saline pre-treated inflamed; Fig 3). Further, induction of cystitis with CYP per se increased the release of NO (from 25.86±1.96 in saline pre-treated controls to 42.99±5.51 µM in saline pre-treated inflamed; Fig 3).

Figure 2. Volume per micturition and voiding frequency in healthy (control) rats and rats treated with CYP in order to induce cystitis. In both groups, animals were pre-treated for 10 days with either saline (1 mL/kg/day s.c.) or a combination drug treatment (B, D; mirabegron 0.6 mg/kg/day s.c. and tolterodine 0.05 mg/kg/day s.c.) against OAB consisting of the muscarinic antagonist tolterodine (0.05 mg/kg/day s.c.) and the β3-adrenoceptor agonist mirabegron (0.6 mg/kg/day s.c.). Induction of cystitis caused (A) a decrease in micturition volume and (B) an increase in frequency which was normalized by OAB drug treatment. Statistical comparisons were made by two-way ANOVA for multiple comparisons. n = 8 in all groups. *p<0.05.

Figure 3. Levels of nitric oxide (NO) in urine from healthy (control) rats and rats treated with CYP in order to induce cystitis. In both groups, animals were pre-treated for 10 days with either saline (1 mL/kg/day s.c.) or a combination drug treatment (B, D; mirabegron 0.6 mg/kg/day s.c.) against OAB consisting of the muscarinic antagonist tolterodine (0.05 mg/kg/day s.c.) and the β3-adrenoceptor agonist mirabegron (0.6 mg/kg/day s.c.). Both combination drug treatment and cystitis caused a significant increase in the levels of NO. Mesmeric antagonism most likely inhibits release of nitric oxide, if anything.1 This is highly interesting in the context of understanding the mechanisms underlying the beneficial effect of mirabegron. However, it should be noted that inflammation per se increases the levels of NO and the level of NO is not significantly different between saline pre-treated and drug pre-treated rats. This indicates that inflammation increases the release of NO, if anything.1

Discussion

Induction of cystitis with CYP alters the micturition pattern, causing a state of overactivity. This is in line with previous studies.2 The current data show that the altered micturition parameters were normalized by a combination therapy which is suggested as the golden standard drug therapy against OAB. The drug combination does not, however, affect healthy bladder parameters. This study does not take side effects into account, but clearly shows the beneficial effects of the combination therapy during cystitis. It is evident that treatment with a combination of tolterodine and mirabegron increases the release of nitric oxide. This needs to be argued to be mainly caused by mirabegron, since muscarinic antagonism most likely inhibits release of nitric oxide, if anything.1 This is highly interesting in the context of understanding the mechanisms underlying the beneficial effect of mirabegron. However, it should be noted that inflammation per se increases the levels of NO and the level of NO is not significantly higher when comparing levels of NO during cystitis with and without drug pre-treatment. Tentatively, this is an indication of maximum NO synthase activity.

Conclusions

Induction of inflammation by cyclophosphamide alters micturition parameters. Combination drug therapy against OAB can normalize the altered parameters. Further, treatment with the combination therapy increases release of nitric oxide in the urinary bladder. However, it remains to be concluded if this significantly contributes to the beneficial effects of, mainly, mirabegron.

References


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