Objectives: The objective was to investigate the influence of two anticholinergic, propiverine and tolterodine on bladder contraction in a standardized in vivo model taking neurogenic innervation into account. In addition, standardized salivary flow measurements enabled the evaluation of hyposalivation, the most predominant anticholinergic side effects. Furthermore, the detrusor electromyogram was evaluated.

Material & Methods: 10 male mini pigs were anaesthesized. The carotic artery was cannulated for blood pressure control and the jugular vein for administration propiverine 0.4 mg/kg b.w and L(+)-tolterodine 0.06 mg/kg b.w. Doses were chosen according to the Defined Daily Dose (DDD). For stimulation-induced salivary flow measurements both lingual nerves were exposed and a cuff electrode was placed around the nerves. The bladder was then exposed and 2 electrodes were implanted under the serosa of the bladder dome to record the bladder EMG and a cystostomy was performed to assess cystometrographic measurements. A bilateral ureterocutaneostomy was performed in order to maintain a constant bladder volume during the trial. The urethra was then ligated to initiate isovolumetric bladder contractions during sacral anterior root stimulation (SARS).

Results: In all experiments, for each animal reproducible intravesical pressure values (pves) and salivary flow rates were elicted during electrostimulation before administration of the drug. Bladder pressure (tab.): After administration of *propiverine*, neurostimulation-induced rise in pves dropped about 64% from the initial value. After administration of *tolterodine* pves declined by about 60%. Salivation: After *propiverine* salivary flow dropped about 61%. Inhibition of salivary flow under *tolterodine* was about 54%. Similar results were obtained in bladder EMG recordings. Both propiverine and tolterodine increased the heart rate temporarily at an median of 18%. No change in ECG recordings was noted after administration of the test substances.

Conclusion: 1. The presented model allows comparative in vivo studies of pharmacological effects on bladder function. **2.** Both drugs have similar effects on bladder inhibition in mini pigs. **3.** Hyposalivation was comparable in both drugs. **4.** The NANC innervation of the mini pig is about 10 to 15%.

propiverine hydrochloride				*tolterodine*			
No.	before [cm H2O]	after [cm H2O]	atropine [cm H2O]	**No.**	before [cm H2O]	after [cm H2O]	atropine [cm H2O]
1	50	18	3	**6**	47	26	4
2	62	6	4	**7**	54	14	7
3	25	17	4	**8**	31	6	2
4	37	10	4	**9**	30	12	6
5	36	33	12	**10**	59	57	19
median	*37*	*17*	*4*		*47*	*14*	*6*

Tab: Results of the stimulation-induced bladder pressure just before and 40 minutes after administration of propiverine hydrochloride (No. 1 to 5) and tolterodine (No. 6-10) as well as after administration of atropine at the end of the trial.

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S. Yamada, A. Tohma, T. Oki, R. Kimura, A. Kawashima*, and M. Uchida*

Dept. of Biopharm., Sch. of Pharm. Sci., Univ. of Shizuoka, Shizuoka, * Dept. of Pharm. Develop., Meiji Milk Products Co. Ltd., Odawara, Japan

RATIONALE FOR THE USEFULNESS OF TRANSDERMALLY ADMINISTERED OXYBUTYNIN IN THE THERAPY OF DETRUSOR INSTABILITY, BASED ON THE ANALYSIS OF MUSCARINIC RECEPTOR BINDING

Aims of Study. Antimuscarinic agent, oxybutynin, is widely used for the treatment of detrusor

instability (DI) or detrusor hyperreflexia which is characterized by symptoms of increased frequency of micturition and urge urinary incontinence [1]. However, the use of this drug is often limited by systemic side effects such as dry mouth known to occur frequently with orally administered oxybutynin. The incidence of dry mouth is expected to be lower with transdermal application of oxybutynin than oral administration. The transdermal therapeutic system (TTS) of oxybutynin (MM-801) has been currently developed and its usefulness is now under investigation. The therapeutic effect and dry mouth by oxybutynin in patients with DI are mainly based on the blockade of muscarinic receptors in the bladder and salivary gland, respectively. The analysis of drug-receptor binding in different tissues in relation to the pharmacokinetics is very useful in characterizing pharmacological specificity (potency, duration of action and tissue selectivity) of the drug [2]. To clarify the usefulness of transdermally administered oxybutynin in the therapy of DI, therefore, we characterized muscarinic receptor binding in rat tissues after the transdermal application of MM-801 in comparison with that after the oral administration of oxybutynin.

Methods. At 1 to 48 hr after the oral and transdermal administration of oxybutynin, rats were sacrificed by exsanguination from the descending aorta, and the bladder, submaxillary gland, heart and colon were dissected. The mucarinic receptor in each tissue was measured by a radioreceptor binding assay with [N-methyl-^{3}H]scopolamine (NMS) as a radioligand, and binding constants of apparent dissociation constant (Kd) and maximal number of binding sites (Bmax) for [^{3}H]NMS were estimated by Scatchard analysis [3,4]. The concentration of oxybutynin and its active metabolite, *N*-desethyloxybutynin (DEOB) in the plasma was also measured.

Results. Following the oral administration of oxybutynin (127 μmol/kg), there was a significant increase in Kd value for specific [^{3}H]NMS binding in the bladder, submaxillary gland, heart and colon of rats compared with the value of control rats, and a concomitant reduction of Bmax value only in the submaxillary gland and heart. Such increase in Kd value in each tissue was seen at 1 and 3 hr after the oral administration of oxybutynin, but not at 12 and 24 hr. In contrast, a significant reduction of Bmax value in the submaxillary gland and heart was maintained for at least 24 hr. The plasma concentration of oxybutynin and DEOB was maximal at 1 hr after the oral administration and it was extremely low at 12 and 24 hr later.

The transdermal application of MM-801 (one patch containing 33.6 μmol oxybutynin base/body) for 2, 4, 12, 24 and 48 hr brought about a significant increase in Kd value for specific [^{3}H]NMS binding in the bladder, submaxillary gland, heart and colon of rats, and there was little reduction of Bmax value in each tissue. The increment of Kd value increased with the application time of MM-801, being maximal at 12 hr later. The plasma concentration of oxybutynin increased with the application time, and the maximal level was seen at 12 hr and maintained until the application time of 48 hr. DEOB was not detected in the plasma of MM-801-administered rats. In rats at 8 hr after the removal of MM-801 following the 48 hr-application, the increase in Kd value for [^{3}H]NMS binding in each tissue was no longer seen and the plasma concentration of oxybutynin was markedly low.

Conclusions. These data suggest that the transdermally administered oxybutynin (MM-801) binds significantly to the muscarinic receptor in the bladder of rats and the binding to the receptor in the submaxillary gland is easily reversible by this TTS but not by the oral administration. Thus, the present study may provide a rationale for the usefulness of tansdermal application of oxybutynin in the therapy of DI.

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Author(s):
H.Nagata, H.Asakura, J.Nakashima, K.Nakamura, M.Murai

Institution, city, country:
Department of Urology, School of Medicine, Keio University, Tokyo, Japan

Title (type in CAPITAL LETTERS, leave one blank line before the text):
BIOLOGICAL CHARACTERIZATION OF ADENOSINE RECEPTORS IN RAT DETRUSOR SMOOTH MUSCLE

Aims of Study: The function of detrusor smooth muscle is mainly regulated by cholinergic and adrenargic receptors in normal conditions. However, non-cholinergic and non-adrenergic regulators of the detrusor function have not been fully elucidated. The present study was undertaken to investigate the biological role of adenosine receptors in the regulation of detrusor function.

Methods: Detrusor muscle strips of 10 x 2 mm of Male Sprague-Dawley rats (weighning 250 to 300 grams) were mounted in an organ bath containing Krebs-Henseleit solution and gassed with 95% O_2, 5% CO_2 and maintained at 37 centigrade. A resting tension of 1 g was applied to the muscle strips and was equilibrated for 60 minutes. The isometric effects of adenosine agonists on detrusor contraction induced by calbachol were measured by force transducer. To measure intracellular cyclic AMP contents, muscle strips were incubated in an organ bath containing Krebs-Henseleit solution and gassed with 95% O_2, 5% CO_2 at 37 centigrade for 30 minutes. Drugs were added and the incubation were given for 5 minutes. After incubation with drugs, muscle strips were rapidly frozen with liquid nitrogen. Frozened muscle strips were added 2 ml of 6% trichroloacetate and homogenized. Afetr samples were centrifuged at 3000g for 10 minutes at 4 degree, the supernatant was removed and added 6 ml of diehtylether. Its content was measured using cyclic AMP assay kit.

Drug: N6cyclo-pentyl-adenosine (CPA), 5N-ethylcarboxamide-adenosine (NECA), N^6cyclo-pentyl-adenosine (CPA), N^6-3-iodo-bendyl-adenosine-5N-methyluronamide (IB-MECA), 8-phenyl-theophylin (8-PT), carbachol chloride (CCh), Forskolin.

Results: The preincubation of 5N-ethylcarboxamide-adenosine (NECA), an adenosine A2 agonist, of 0.01 to 10 mM inhibited the contraction of the muscle strips induced by carbachol in a dose-dependent manner. NECA also produced significant increases in intracellular cyclic AMP levels of the muscle strips in a dose-dependent manner(Fig.1). The relaxation of the muscle strips and the elevation of intracellular cyclic AMP levels induced by 0.1 mM NECA were significantly inhibited
by 1 mM 8-phenyl-theophyline, an adenosine receptor antagonist. On the other hand, N^6cyclo-pentyl-adenosine, an adenosine A1 agonist, and N^6-3-iodo-bendyl-adenosine-5N-methyluronamide, an adenosine A3 agonist, at a concentrated range of 0.01 to 10 mM did not have any effects on the contraction of the muscle strips induced by carbachol.

Conclusions: These data demonstrate that the adenosine A2 agonist significantly inhibited the detrusor contraction induced by the cholinergic