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IN VIVO DEMONSTRATION OF MUSCARINIC RECEPTOR SELECTIVITY OF IMIDAFENACIN IN MOUSE TISSUES.

Hypothesis / aims of study

Imidafenacin, a potent and selectice antagonist of M_1 and M_3-muscarinic receptor subtypes, is now used clinically in Japan for the treatment of overactive bladder (OAB) [1-3]. Pharmacological studies of this agent showed selectivity in the bladder over salivary gland and brain. Oral administration of imidafenacin at low doses caused a more selective and longer-lasting binding to muscarinic receptors in the bladder than at other tissues such as the salivary gland, heart, colon, lung and brain, suggesting preferential muscarinic receptor binding in the bladder [2,3]. Pharmacokinetics data showed that the orally administered imidafenacin distributed at a higher concentration in the bladder than the serum or submaxillary gland of rats. The present study was undertaken to characterize the in vivo muscarinic binding of imidafenacin in mouse tissues by using a tritiated ligand with high specific acitivity.

Study design, materials and methods

[^{3}H]imidafenacin (851 GBq/mmol, 12 nmol/kg) was injected in to the tail vain. The mice were sacritificed under anesthesia with isoflran at 10, 30, 90 and 180 min. A blood sample was taken from the descending aorta, tissues (bladder, submaxillary gland, heart, colon, lung and cortex) were rapidly removed. After dissection on ice, each tissue was homogenized in ice-cold 50 mM Na^+/K^+ phosphate buffer to give a final tissue concentration of 20 mg/mL using Polytron homogenizer. Particulate-bound radioactivity was determined by rapid filtration of 0.5 mL of homogenate over Whatman CF/C filters, which were washed subsequently with 1 mL of ice-cold buffer. Radioactivity was measured in a liquid scintillation counter. Based on the data on pharmacological specifity, the particulate-bound radioactivity from vehicle- and atropine (5 mg/kg i.p.)-pretreated rats was defined as total binding and nonspecific binding, respectively, and the difference was taken as the in vivo specifc binding of [^{3}H]imidafenacin.

Results

Pretreatment with atropine reduced the [^{3}H]imidafenacin binding in particulate fractions of the bladder, submaxillary gland, heart and colon. Therefore, as shown in fig. 1, there was a significant the difference in particulate-bound radioactivity of [^{3}H]imidafenacin in the bladder. Submaxillary gland, heart and colon between vehicle- and atropine-pretreated mice.

There were notable differences among tissues in the time course (10 to 180 min) of specific [^{3}H]imidafenacin binding after i.v. injection of the ligand. The specific binding in the submaxillary gland, heart, colon and lung was greatest at 10 min. The specific binding in the heart, colon and lung declined rapidly with the disappearance of [^{3}H]imidafenacin from the plasma. On the other hand, [^{3}H]imidafenacin binding in the bladder attained peak levels at 30 min, and the degree of binding was sustained until 90 min or decreased gradually, with considerable binding remaining even at 180 min(fig. 2).

Interpretation of results

The present study shows that imidafenacin binds to the muscarinic receptor and exerts more selective and longer-lasting effect on the bladder than other tissues, such as the submaxillary gland, heart, colon, lung and cortex in vivo experiment.

Concluding message

It is concluded that [^{3}H]imidafenacin labels selectively bladder muscarinic receptors in vivo. Thus, the present study may provide a rationale for the pharmacological usefulness of imidafenacin as therapeutic agent of overactive bladder. Also, this radioligand may be useful to characterize muscarinic receptors in tissues such as the bladder.

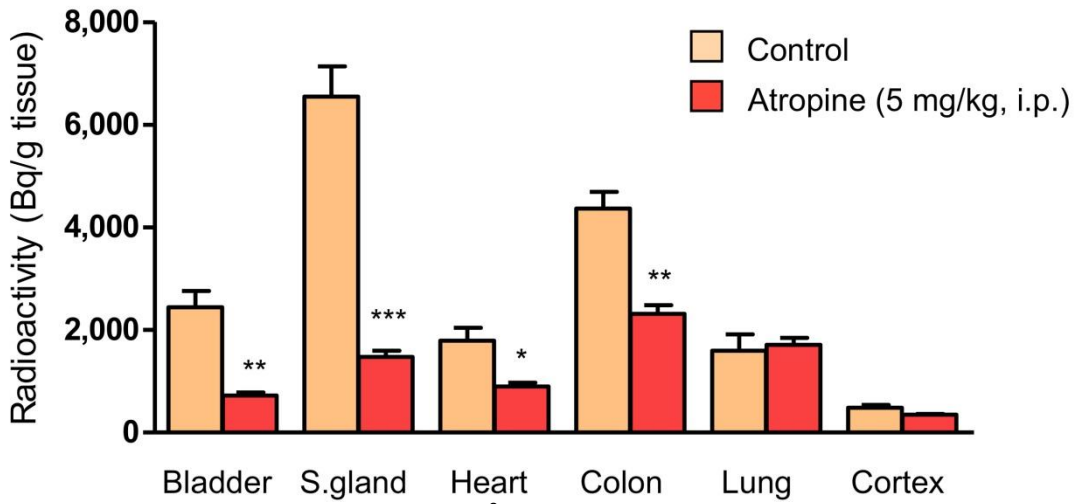

Fig. 1 Effect of pretreatment with atropine on [^{3}H]Imidafenacin binding in mouse tissues. Mice received saline (control) and atropine (5 mg/kg) at 30 min prior to i.v. injection of [^{3}H]Imidafenacin. [^{3}H]Imidafenacin (12 nmol/kg) was injected into tail vein,

and mice were sacrificed at 30 min, and [^{3}H]Imidafenacin binding in particulate fractions of each tissue was determined. Each column represents mean±S.E. of 4 mice. Asterisks show a significant difference from control values, *P<0.05, **P<0.01, ***P<0.001.

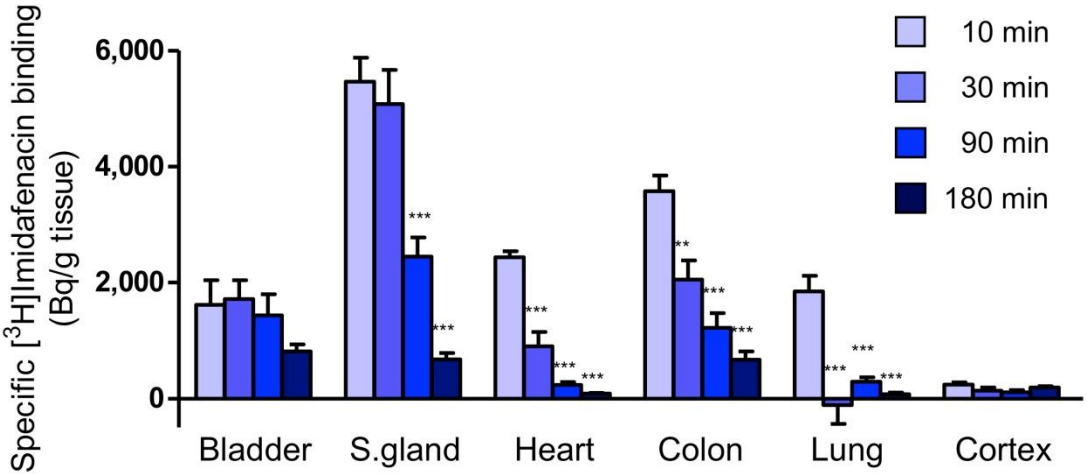

Fig. 2 Time course of in vivo specific binding of [^{3}H]Imidafenacin in mouse tissues after i.v. injection of the ligand. [^{3}H]Imidafenacin (12 nmol/kg) was injected into tail vein, and mice were sacrificed 10, 30, 90 and 180 min later. Specific [^{3}H]Imidafenacin binding was experimentally defined as the difference in binding in particulate fractions of each tissue saline (total binding)- and atropine (5 mg/kg, i.p.) (nonspecific binding)-pretreated mice. Each column represents mean±S.E. of 4 to 6 mice. Asterisks show a significant difference from each value at 10 min, **P<0.01, ***P<0.001.

References

1. Azneimittelforschung 57: 92-100, 2007.
2. J Pharmacol Sci 112: 142-150, 2010.
3. J Pharmacol Exp There 336: 365-371, 2011.

Disclosures

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