Effect of muscarinic receptor antagonists on contractions of circular muscle in human colon

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Muscarinic receptors are commonly expressed in the digestive tract. Today, muscarinic receptors include several subtypes, already characterized in several animal models. However, the functional role of M_1, M_2, M_3 and M_4 subtypes in human colon is poorly defined (1-2). Therefore, we started a pilot study testing the effect of selective muscarinic receptor antagonists (M_1-$M_{4)}$ on betanechol-evoked contractions in human colonic circular muscle. **METHODS**. Segments of sigmoid colon were obtained from 6 male patients (mean age 74.8yrs, range 68-80yrs) undergoing left hemicolectomy for non-obstructive sigmoid cancer according to a protocol approved by the local ethics committee. Colonic circular muscle strips were taken from macroscopically normal areas. Strips (15x3mm; deprived of the mucosa) were mounted isometrically in an organ bath with oxygenated Krebs solution at 37° C and placed under a tension of 20-24mN. After a 60-min stabilization period, at least two comparable response to carbachol (10^{-4}M) were recorded before studying the response to muscarinic receptor antagonists. Cumulative concentration-response curves were obtained with betanechol (10^{-8}-10^{-2}M) in the absence and in the presence of M_1-M_4 antagonists (10^{-9}-10^{-5}M) added 30 min before betanechol curves. The following antagonists were tested: pirenzepine dihydrochloride (M_1), AF-DX116 (M_2), 4-DAMP (M_3), PD 102807 (M_4). EC_{50} values were calculated from log concentration-response curves, and 95% confidence intervals (CIs) were calculated using nonlinear regression. **RESULTS**. All strips contracted concentration-dependently when exposed to betanechol. The lowest effective concentration for M_1, M_2 and M_4 receptor antagonists was 10^{-6}M, whereas for the M_3 receptor antagonist it was 10^{-8}M. EC_{50} value for betanechol was 4.43 x10^{-5}M (95% CI 2.10-9.35x10^{-5}M). EC_{50} values for M_1, M_2, M_3 and M_4 were 3.06x10^{-4}M (95% CI 2.54-3.7x10^{-4}M), 1.34x10^{-4}M (95% CI 3.67x10^{-5}M-4.87x10^{-4}M), 4.22x10^{-4}M (95% CI 3.38-5.27x10^{-4}M), 1.17x10^{-4}M (95% CI 7.42x10^{-5}M-1.84x10^{-4}M) respectively. The shift to the right of the betanechol concentration-response curve was statistically significant in the presence of the M_1 and M_3 receptor antagonist. **CONCLUSION.** These preliminary data indicate that, in elderly male patients, the most representative muscarinic receptor mediating bethanechol-induced contraction is the M_3 subtype. Further studies aimed at characterizing possible age- and gender-dependent differences are warranted to optimize the approach to smooth muscle motility disorders.

(1) Harrington AM et al. 2010 Neurogastroenterol Motil 22, 999-1007
(2) Krueger D et al. 2013 Neurogastroenterol Motil May 19. doi: 10.1111/nmo.12156.