Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue3abst043P.pdf

Bacterial-derived gamma-aminobutyric acid significantly attenuates proliferation of human colon adenocarcinoma cells in vitro

Florian Kratz[1,2], Sinead Heuston[1,2], Lis London[2,3], Paul Ross[2,3], Catherine Stanton[2,3], Aileen Houston[1,2], Niall Hyland[1,2]. [1]University College Cork, Cork, Ireland, [2]Alimentary Pharmabiotic Centre, Cork, Ireland, [3]Teagasc Food Research Centre, Moorepark, Ireland

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. However, GABA and its receptors have recently emerged as having potential tumour-suppressive effects, with the strongest evidence found in colon cancer. Previously we demonstrated that GABA significantly reduced the proliferation and migration of human and mouse colon cancer cells *in vitro* (Fernandes et al., 2012). Lactic acid bacteria have been described as *cell factories* for GABA (Li & Cao, 2012), and thus have potential as therapeutic tools for the delivery of anti-cancer agents to the colon. However, the ability of GABA-producing bacteria to suppress colon cancer is unknown. Thus, the aim of the current study was to investigate the effect of bacterial-derived GABA on the proliferation of human (SW480) and murine (CT26) colon tumour cell lines *in vitro*. A screen of 91 human intestinally-derived bacteria identified five efficient GABA-producing strains (Barret et al., 2012). Of these, we selected a Lactobacillus with the greatest GABA production in the presence of monosodium glutamate (MSG) to prepare GABA-enriched supernatants. Control supernatants were prepared by omitting MSG from the culture media. Lyophilised supernatants from Lactobacilli cultured with MSG contained 0.858 mg/mg GABA, while those prepared from Lactobacilli cultured in the absence of MSG contained 0.015mg/mg GABA. SW480 and CT26 cells were cultured under standard conditions and treated with GABA (10µM), GABA-enriched (final concentration of GABA, 1µM) and control supernatants for 16 hours, after which cell proliferation was measured by resazurin reduction. To determine whether the anti-proliferative effect of GABA (10µM) on SW480 cells was mediated by $GABA_A$ or $GABA_B$ receptors, cells were pre-treated (1 hour) with bicuculline (100µM) or phaclofen (100µM) respectively, and incubated overnight with GABA in the presence of the antagonists. Data are representative of three independent experiments performed in triplicate (supernatant studies) or two independent experiments performed in duplicate/triplicate (antagonist study). Statistical differences were determined using a one-way ANOVA. All tests were performed using GraphPad Prism 5. Both GABA (10µM; $p<0.05$) and GABA-enriched bacterial supernatants ($p<0.05$) significantly inhibited proliferation of the human adenocarcincoma cell line, SW480. In contrast, supernatants obtained from Lactobacilli cultured in the absence of MSG (control) had no significant effect on proliferation. Neither GABA (10µM), nor either of the bacterial-derived supernatants, significantly influenced proliferation of murine, CT26 colon tumour cells. This is consistent with our previous findings with GABA (500nM-100µM) in which GABA had the greatest inhibition of proliferation in the range 2.5µM-5µM (Fernandes et al., 2012). Neither the $GABA_A$ nor $GABA_B$ receptor antagonists significantly inhibited the anti-proliferative effect of GABA on SW480 cells. These data demonstrate that bacterial-derived GABA exerts

an anti-proliferative effect on human adenocarcinoma cells *in vitro*, and suggests that further characterisation of GABA production by such bacteria, and their application in preclinical models of colon cancer is warranted.

Fernandes P et al, Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst044P.pdf, 2012

Li H & Cao Y, Amino Acids 39:1107, 2010.

Barrett E et al, J Appl Microbiol 113:411, 2012.