Intestinal mucositis induced by 5-FU results in glial changes modified by analgesics via neuro-immune mechanisms

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BACKGROUND

- Analgesic choice in oncology is problematic due to exacerbating gastrointestinal symptoms
- Chemotherapy drugs induce **intestinal mucositis**[1], **pain**[2] & often **cognitive impairment** (CICI)[3]
- Neuro-immune glial cells, **microglia** and **astrocytes** are sensitive to peripheral inflammatory events & analgesics, modifying pain signalling and cognition[4,5]
- Glial reactivity may occur **directly** via central insults or **indirectly** via peripheral-to-central neuro-immune signalling pathways; cellular, humoral or neural pathways[6]

Research problem

- Chemotherapy induces intestinal mucositis, pain & CICI
- Whilst analgesics ameliorate pain, they may exacerbate chemotherapy-induced gut side-effects
- Analgesics may also modulate inflammatory responses and glial cell expression via neuro-immune signalling pathways

Hypothesis

- Glial changes will contribute to pain signalling and affect higher order brain regions involved in cognition via neuroimmune signalling mechanisms in rats with intestinal mucositis

Aims

- Characterise the effect of 3 analgesics on acute intestinal inflammation and neuro-immune mechanisms in a rat model of 5-fluorouracil (5-FU)-induced intestinal mucositis
- Assess acute intestinal mucositis via myeloperoxidase (**MPO**) activity in the jejunum & ileum of rats
- Quantify glial reactivity & pro-inflammatory response, determining neuro-immune pathway via a) humoral: *hippocampal* or b) neuronal: *thoracic* (T6-T9; innervated by small intestine)

METHODS

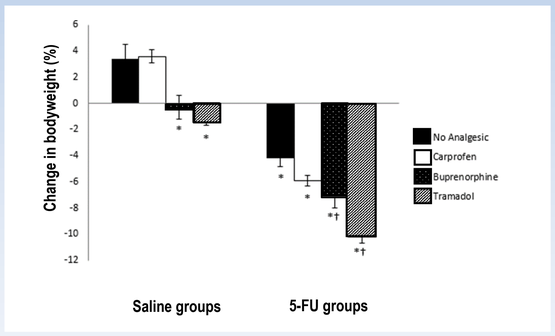
- Female DA rats (n=8)
- I.P. administration of either saline or 5-FU (150mg/kg) in combination with 12 hourly doses of opioid derivatives (buprenorphine; BUP, 0.05kg/mg or tramadol; TRAM, 12.5mg/kg) or NSAID (carprofen; CARP, 15mg/kg)
- Rats were humanely euthanized at peak injury phase of intestinal mucositis (72h after saline or 5-FU administration)
- Intestinal sections were quantified using MPO assay & CNS sections using western blot staining for microglia (CD11b), astrocyte (glial fibrillary associated protein; GFAP) & interleukin-1 beta (IL-1β)
- All results $p < 0.05$ deemed significant

REFERENCES

1. Sonis 2004. Nat Rev Cancer 4, 277-284; 2. McGuire *et al.* 1993. Oncol Nurs For 20, 1493-1502; 3. Wigmore *et al.* 2010. Adv Exp Med Biol 678, 157-164; 4. Parpura *et al.* 2012. J Neurochem 121, 4-27; 5. Grace *et al.* 2014. Nat Rev Immunol 14, 217-231; 6. Dantzer *et al.* 2010. Auton Neurosci 85, 60-65.

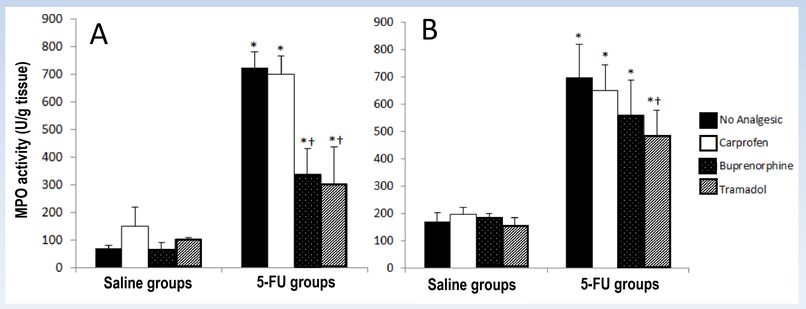
RESULTS

Figure 1. Change in bodyweight (%)



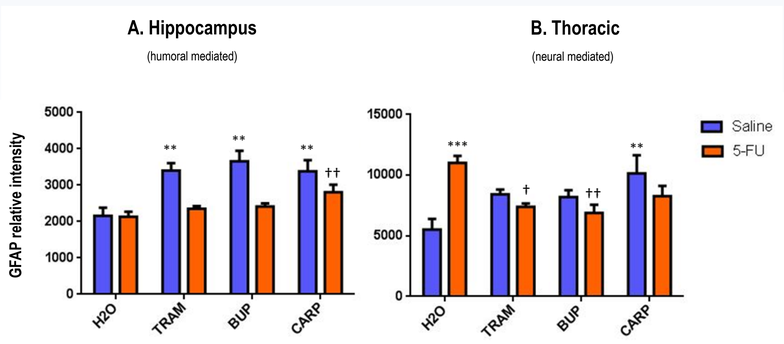
Effect of analgesic agents on bodyweight change 72 hr after either saline or 5-FU injection. * indicates $p < 0.05$ compared to saline + no analgesic and † $p < 0.05$ compared to 5-FU + no analgesic

Figure 2. Acute intestinal inflammation changes



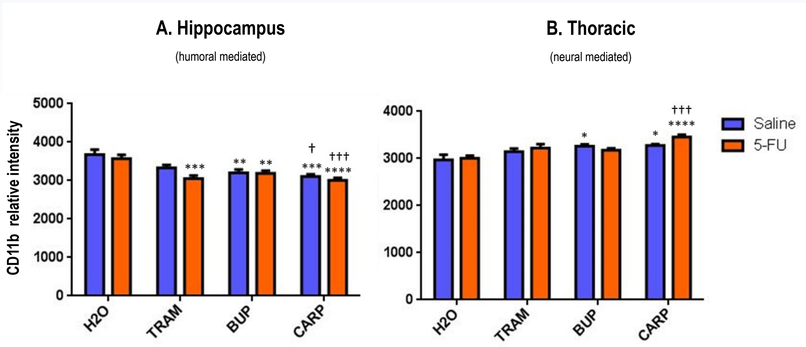
Effect of analgesic agents on myeloperoxidase (MPO) activity in the jejunum (A) and ileum (B) 72 hr after either saline or 5-FU injection. Data are expressed as mean (MPO units/g tissue) ± SEM. * indicates $p < 0.05$ compared to saline + no analgesic and † $p < 0.05$ compared to 5-FU + no analgesic

Figure 3. Astrocyte (GFAP) expression changes



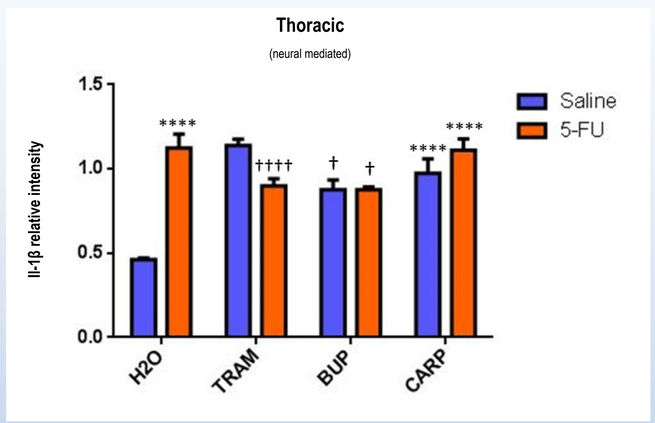
Effect of analgesic agents on GFAP (astrocyte) expression in the hippocampus (A) and thoracic (B) 72 hr after either saline or 5-FU injection. Data are expressed as mean (relative intensity compared to β-actin) ± SEM. * indicates $p < 0.05$ compared to saline + no analgesic and † $p < 0.05$ compared to 5-FU + no analgesic

Figure 4. Microglial (CD11b) expression changes



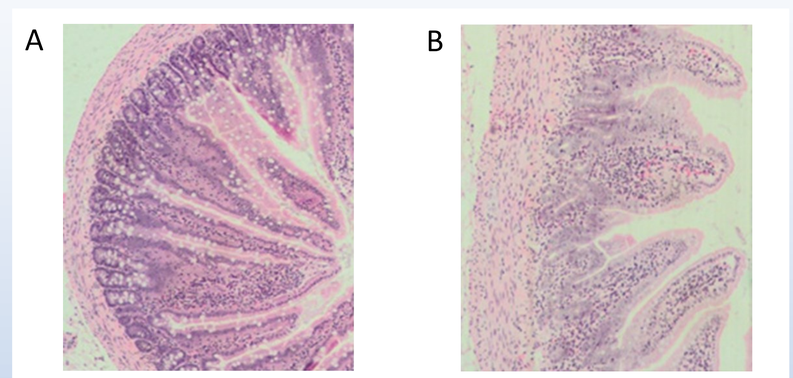
Effect of analgesic agents on GFAP (astrocyte) expression in the hippocampus (A) and thoracic (B) 72 hr after either saline or 5-FU injection. Data are expressed as mean (relative intensity compared to β-actin) ± SEM. * indicates $p < 0.05$ compared to saline + no analgesic and † $p < 0.05$ compared to 5-FU + no analgesic

Figure 5. CARP & BUP attenuate thoracic IL-1β expression increase following 5-FU exposure



Effect of analgesic agents on CD11b (microglial) expression in the hippocampus (A) and thoracic (B) 72 hr after either saline or 5-FU injection. Data are expressed as mean (relative intensity compared to β-actin) ± SEM. * indicates $p < 0.05$ compared to saline + no analgesic and † $p < 0.05$ compared to 5-FU + no analgesic

Figure 6. Jejunal histological severity score of saline VS 5-FU control



Representative photomicrographs (x 100) of the proximal jejunum sections stained with haematoxylin and eosin in animals treated with saline alone (A), 5-FU alone (B)

DISCUSSION

- BUP & TRAM may be more effective analgesic options in 5-FU-induced intestinal mucositis
  - Attenuated acute intestinal inflammation (MPO activity)
  - Increased hippocampal GFAP expression
    - Co-administration of 5-FU returns GFAP increase to basal levels
  - Thoracic IL-1β expression was increased in 5-FU treated rats, yet reduced in opioid analgesics
  - CD11b changes were most evident in hippocampal and thoracic CARP groups
  - Thoracic inflammatory effect was observed in CARP group
- Neuro-immune signalling pathways associated with the glial changes:
  - Humoral (hippocampal) & neural (thoracic) pathways
- 5-FU induced an increase in thoracic GFAP expression (indicative of astrocyte activation), potentially enhancing pain signalling pathways as indicated by elevated IL-1β levels
- Intestinal histological score remained unchanged in opioid groups despite beneficial reduction in MPO activity