EXAMINATION OF LOPERAMIDE AND CROFELEMER IN A RAT MODEL OF NERATINIB-INDUCED DIARRHEA

Joanne Bowen¹, Kate Secombe¹, Imogen Ball¹, Joseph Shirren¹, Anthony Wignall¹, Hannah Wardill², Ysabella Van Seville³, Francesca Avogadri-Connors⁴, Susan Moran⁴, Elizabeth Olek⁴, David Martin⁴

(1) Adelaide Medical School, University of Adelaide, Australia, (2) Centre for Nutrition and Gastrointestinal Diseases, South Australian Health and Medical Research Institute, Australia, (3) Division of Health Sciences, University of South Australia, Australia, (4) Puma Biotechnology, Inc, Los Angeles, USA

CANCER TREATMENT
TOXICITIES GROUP
Integrating the pathogenesis, prediction and prevention of cancer-related side effects

Introduction

Neratinib

- Irreversible small molecule pan-ErbB tyrosine kinase inhibitor
- Approved for adjuvant treatment of HER2⁺ breast cancer
- Associated with 40% grade 3+ diarrhea when no prophylactic anti-diarrheal used¹

Model development

- Our group has established a rat model of neratinib-induced diarrhea to investigate new interventions
- Current standard of care for diarrhea is loperamide
- A novel agent, crofelemer, has been suggested to target mechanisms of diarrhea by preventing epithelial chloride secretion²
- May be a new prophylaxis approach

Figure 1: Receptor tyrosine kinase inhibitor mechanism of action for neratinib

Study Objective

To compare effectiveness of loperamide vs crofelemer for prevention of neratinib-induced diarrhea in a rat model

Specific aims:

- 1) To assess impact on diarrhea incidence and severity
- 2) To determine effect on neratinib absorption
- 3) To quantify changes to intestinal physiology & permeability

Materials and Methods

Animal model

64 male albino Wistar rats treated daily by oral gavage with either:

- 50 mg/kg neratinib
- 50 mg/kg neratinib + 0.4 mg/kg loperamide HCl
- 50 mg/kg neratinib + 25 mg/kg crofelemer
- vehicle control (0.5% HPMC)

Groups of rats (n=4) were killed at 7, 14, 21 and 28 days.

Diarrhea and neratinib analysis

Rats were assessed for **weight loss and diarrhea** daily using an established grading system (0=none, 1=soft, 2=wet, 3=wet with extended fur staining). **Serum neratinib** was measured weekly (C_{min} - 24 h after previous dose) or over 24 h (pharmacokinetic - 0, 1, 3, 6, 9, 12, 24 h) by **LC-MS/TOF**.

Tissue electrophysiological analysis

Fresh ileum and colon tissue was stripped of the muscle layer and mounted in Ussing chambers to assess **permeability and ion conductance**.

Statistical analysis

Repeated measures 2-way ANOVA and Freidman's test: weight gain, serum neratinib and diarrhea incidence and severity.

One-way ANOVA and Kruskal-Wallis test: Permeability and conductance
Data shown as mean +/- SEM unless otherwise stated.

Results

1. No improvement in neratinib-induced diarrhea with loperamide or crofelemer treatment & more pronounced delay in weight gain

2. Loperamide increased serum neratinib concentration at 2 weeks & crofelemer decreased absorption after a single dose

3. Mucosal permeability and ion conductance were not significantly altered by loperamide or crofelemer after 28 days of treatment

Conclusions

- This study found no evidence of effectiveness for the anti-diarrheal agents, loperamide and crofelemer, for prevention of neratinib-induced diarrhea.
- Serum neratinib was increased in rats co-treated with loperamide (303 ng/ml) compared to neratinib alone (198 ng/ml) on day 14 and may be associated with altered metabolism. This coincided with worse average grade diarrhea compared to neratinib alone and crofelemer-treated rats during the first 14 days. Crofelemer was associated with a decrease in 0-24 h AUC (39890 ng*h/ml) compared to neratinib alone (60983 ng*h/ml) after a single dose, but did not alter weekly concentrations. This likely reflects that crofelemer is not systemically absorbed.
- Resistance was not significantly changed indicating that tissue injury did not lead to increased permeability. Non-significant changes in ion conductance suggest that neratinib-induced diarrhea is not driven by a chloride secretory mechanism in our rat model and other mechanisms should be explored.

References: 1. Chan et al. (2016). Lancet Oncol 17: 367-77; 2. Van Seville et al. (2018). Int J Cancer 142:369-80.

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