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VITAMIN D DEFICIENCY EXACERBATES 5-FLUOROURACIL-INDUCED MUCOSITIS IN THE COLON

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INTRODUCTION

- Gastrointestinal mucositis (GM) is a severe, dose-limiting intestinal toxicity of cancer treatments, impacting the intestine through inflammation, apoptosis, loss of intestinal barrier function and gut microbiota dysbiosis ^{3,4}
- Vitamin D deficiency maybe associated with cancer incidence and inflammatory GI conditions, leading to gut microbiota dysregulation
- We aimed to investigate the effect of vitamin D deficiency on the severity of GM in the large intestine of mice, and the effect of vitamin D therapies in ameliorating the effects of GM in both vitamin D-replete and D-deficient mice

METHODS

- C57Bl/6 male mice were fed a diet containing 1000 IU/kg or 0 IU/kg of vitamin D, respectively, for 5 weeks pre-chemotherapy
- 5 days prior to chemotherapy, vitamin D treatments (6.25 mg/kg 25 hydroxyvitamin D [25(OH)D], 500ng/kg VD1-6 [catabolism inhibitor], 25(OH)D + VD1-6 or saline [vehicle control]) were injected subcutaneously daily for seven days into mice.
- 300 mg/kg of 5-fluorouracil (5-FU) or saline (vehicle control) was administered after five days, with mice humanely killed 48 hours later
- Colon sections were stained with H&E, Ki-67 immunohistochemistry (cellular proliferation), Alcian Blue-Periodic acid Schiff's (mucin) and Masson's Trichrome staining (connective tissue) ²
- Real-time PCR determined the relative mRNA expression of toll-like receptor (TLR)4, TLR5, nuclear factor kappa B (NFκB), claudin-1 and -3, vitamin D receptor (VDR), and zonulin-1 (ZO-1)

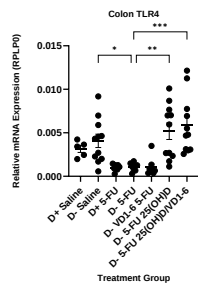


Figure 5. Colon PCR toll like receptor 4 (TLR4) relative mRNA expression

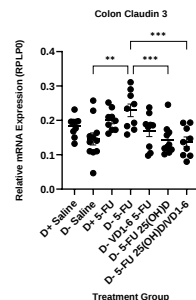


Figure 6. Colon PCR claudin 3 relative mRNA expression

RESULTS

- Colon crypt depth and area significantly increased ($p < 0.05$), substantial damage was observed after vitamin D deficiency (Figure 1)
- Vitamin D deficiency alone significantly reduced cellular proliferation ($p < 0.05$) and increased goblet cell cavitation (Figure 2)
- 5-FU administration significantly reduced cell proliferation ($p < 0.05$) and caused significant structural changes (Figure 2)
- In 5-FU treated mice, vitamin D deficiency further exacerbated damage seen with 5-FU alone (Figure 3 and Figure 4)
- Vitamin D treatments maintained colon crypt depth and area in vitamin D-deficient mice administered 5-FU ($p < 0.05$) (Figure 1 and Figure 3)
- 5-FU administration significantly reduced the relative mRNA expression of TLR4, NFκB, VDR and ZO-1 ($p < 0.05$) (Figure 5), and relative mRNA expression of claudin-3 was significantly increased ($p < 0.05$) (Figure 6)
- The vitamin D treatments maintained claudin-3 expression at control levels, and 25(OH)D/VD1-6 treatment maintained TLR4 expression ($p < 0.05$) (Figure 5 and Figure 6)

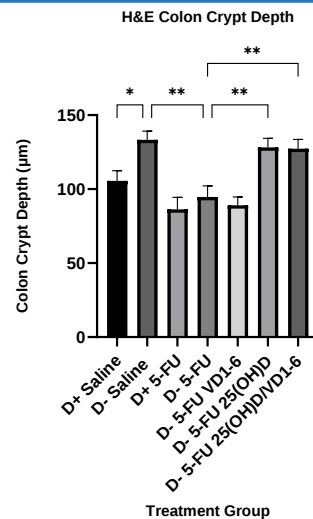


Figure 1. Colon H&E Staining Average colon crypt depth (μm)

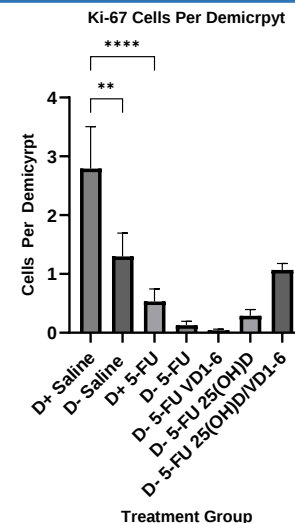


Figure 2. Colon IHC Ki-67 cells per demicrypt counts

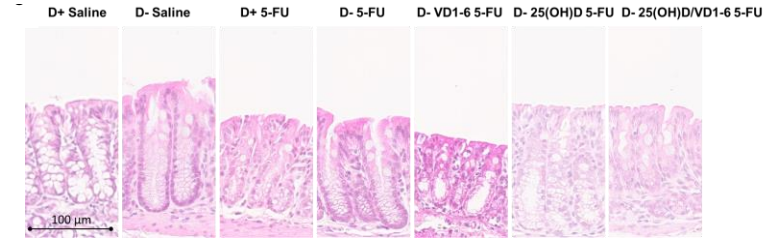


Figure 3. Colon H&E Staining Slide photographs 20x magnification

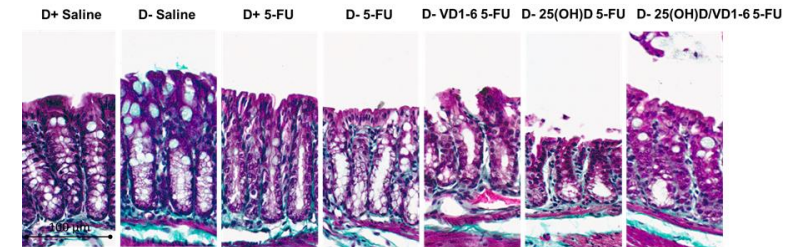


Figure 4. Colon Mason's Trichrome staining slide photographs 20x magnification

CONCLUSIONS

- Vitamin D deficiency exacerbates 5-FU-induced GM in the colon
- Vitamin D-related treatments show potential to alleviate GM

REFERENCES

- Aggelatopoulou, I, Marangos, M, Assimakopoulos, SF, Mouzaki, A, Thomopoulos, K & Triantos, C 2023, 'Vitamin D and Microbiome: Molecular Interaction in Inflammatory Bowel Disease Pathogenesis', Am J Pathol, Mar 1.
- Al-Dasooqi, N, Bowen, JM, Gibson, RJ, Logan, RM, Stringer, AM & Keefe, DM 2011, 'Irinotecan-induced alterations in intestinal cell kinetics and extracellular matrix component expression in the dark agouti rat', Int J Exp Pathol, vol. 92, no. 5, Oct, pp. 357-365.
- Al-Dasooqi, N, Sonis, ST, Bowen, JM, Bateman, E, Blijlevens, N, Gibson, RJ, Logan, RM, Nair, RG, Stringer, AM, Yazbeck, R, Elad, S, Lalla, RV & Mucositis Study Group of Multinational Association of Supportive Care in Cancer/International Society of Oral, O 2013, 'Emerging evidence on the pathobiology of mucositis', Support Care Cancer, vol. 21, no. 7, Jul, pp. 2075-2083.
- Stringer, AM, Hargreaves, BM, Mendes, RA, Blijlevens, NMA, Bruno, JS, Joyce, P, Kamath, S, Lahelji, A, Ottaviani, G, Secombe, KR, Tonkaboni, A, Zadik, Y, Bossi, P & Wardill, HR 2024, 'Updated perspectives on the contribution of the microbiome to the pathogenesis of mucositis using the masc/isoo framework', Support Care Cancer, vol. 32, no. 8, Jul 31, p. 558.