DEVELOPMENT OF A 3D-ORGANOID MODEL OF METHOTREXATE-INDUCED MUCOSITIS

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Introduction

Gastrointestinal mucositis is a side-effect of chemotherapy that causes significant gut toxicity¹. This results in clinical manifestations that affect the course of chemotherapy². Currently, the number of *in vitro* systems suitable to study the mucosal damage are limited. In intestinal *in vitro* organoid cultures, the ability of stem cells to differentiate into specialized intestinal cell types makes this model a powerful tool to study mucositis^{3,4}.

Aim

This study aims at developing a MTX-induced mucositis 3D-organoids model. To validate this model, we characterized the alterations in morphology, cell viability and cytokine production.

Materials and methods

Intestinal organoids derived from mouse ileum were grown for 7 days and incubated with different concentrations of methotrexate (MTX), ranging from 0-1000 ng/ml. Metabolic activity, citrulline levels and cytokine/chemokine production were measured to determine the optimal dosage and incubation time.

Figure 1. Expansion (passaging) and morphology of organoids grown for 7 days. Representative bright-field images of days 1, 3, 5, and 7 after passaging.

Folinic acid (0.0005-50 µg/mL) was added in combination with MTX for different timepoints. To evaluate the effects of short-chain fatty acids in the model, different concentrations of butyrate (0.25-2mM) were added for 96 hours.

Results

MTX affects metabolic activity and citrulline production in a dose-dependent manner

Figure 2. Evaluation of methotrexate-induced cytotoxicity in organoids. Organoids were treated with different concentrations of MTX (0, 10, 100 and 1000 ng/mL) for 96 hours (A). Relative metabolic activity (B) and the levels of citrulline (C) were measured at 24, 48, 72 and 96 hours. All data are presented as mean ± SEM. * (p<0.05), ** (p<0.01) and *** (p<0.001) represent the significant changes between MTX-treated and untreated organoids.

Folinic acid reverts MTX effect in the organoids in a dose-dependent manner

Figure 5. Relative metabolic activity of MTX and FA-treated organoids at different stages of treatment. Organoids were treated with MTX and at different time points 5 µg/mL FA were added to the cultures. Metabolic activity was measured by WST-1 assay. All data are presented as Mean ± SEM. ##### p<0.0001 represents the significant differences observed between untreated organoids and MTX-treated organoids. **p<0.01 and p<0.0001 represent the differences observed between MTX-treated organoids and MTX/FA-treated organoids.

MTX induces inflammation in the MTX-induced mucositis organoids

Figure 3. Cytokine and chemokine production in the MTX-induced mucositis organoids. Organoids were treated with MTX and incubated for 96 hours. At different timepoints supernatant was collected and CXCL10 (A) and TNF-α (B) were measured by Multiplex Immunoassay. All data are presented as mean ± SEM. * (p<0.05), ** (p<0.01) and represent the significant changes between the MTX-treated and untreated groups.

MTX-treated organoids metabolism is fully recovered after refreshment of media

Figure 4. Relative metabolic activity after media refreshment. After 96 hours of incubation, medium containing MTX was washed away and new media was added and at 24, 48, 72 and 96 hours WST-1 was performed. All data are presented as mean ± SEM. * (p<0.05), ** (p<0.01) and *** (p<0.001) represent the significant changes between MTX-treated and untreated groups.

Butyrate increases metabolic activity in MTX-treated organoids

Figure 6. Relative metabolic activity of MTX-treated organoids after butyrate administration. Organoids were treated with MTX and different doses of butyrate for 96 hours. Relative metabolic activity was measured by WST-1. All data are presented as Mean ± SEM. #p<0.05, ####p<0.001, ##### p<0.0001 represents the significant differences observed between untreated organoids and butyrate-treated organoids. **p<0.01 and ***p<0.0001 represent the differences observed between MTX-treated organoids and MTX/butyrate-treated organoids.

Conclusions

- MTX causes significant organoid damage, which can be reverted upon removal of MTX;
- The protective effects of folinic acid suggest that the model is clinically relevant;
- Treatment with butyrate might be a valuable strategy for mucositis treatment;
- This model can potentially be used to test new interventions that aim at reducing mucositis severity.

References

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