Use of *in vitro* Reconstructed Intestinal Epithelium to Study Effects of Repeated Aluminum Exposure

G. De Negri Atanasio[1], G. Allaria[1], L. Dondero[1], F. Rispo[1], F. Tardanico[1], E. Lertora[1], K. Cortese[2], S. Ferrando[1], F. Soggia[3], J. Markus[4] , S. Letasiova[4] ,T. Filippini[5], F. Robino[6], M. Zanotti Russo[6], M. Klausner[7], S. Ayehunie[7], E. Grasselli[1,8] [1]DISTAV, University of Genoa, 16132 Genoa, Italy, [2]Cellular Electron Microscopy Laboratory, DIMES, Human Anatomy, University of Genoa, 16132 Genoa, Italy, [3]DCCI, University of Genoa, Via Dodecaneso 31, 16146 Genoa, Italy, [4]Mattek - Now part of Sartorius, Bratislava, Slovakia, [5]CREAGEN—Environmental, Genetic and Nutritional Epidemiology Research Center, Section of Public Health, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy, [6]Angel Consulting Via San Senatore 14, 20122 Milano, Italy, [7]Mattek - Now part of Sartorius, Ashland, MA USA, [8]Centro 3R, Interuniversity Centre for the Promotion of 3Rs Principles in Teaching and Research, Italy

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Abstract

Background and Objectives Aluminum (Al^{3+}) is one of the most prevalent elements in the environment and is commonly found in dietary products. The free cation, Al^{3+}, is physiologically reactive and thus may affect metabolism when ingested. The gastrointestinal tract plays a pivotal role in its absorption, and it is estimated that approximately 40% of ingested aluminum is retained in the intestinal tissues. The present study aims to evaluate morphological alterations, barrier effects, and gene expression pattern changes after repeated exposure of *in vitro* intestinal tissue models to aluminum.

Materials and Methods EpiIntestinal™ Tissue Preparation: Small intestine (SMI) epithelial cells were harvested using proprietary techniques from post-mortem donors following IRB approval. The tissues were produced by seeding the SMI epithelial onto tissue culture inserts, raising them to the air liquid interface, and culturing them for 2 weeks in specially formulated culture medium to induce in-vivo-like differentiation.

Al^{3+} was applied twice a day for two hours at 5, 20, and 50 ppm for 12 days onto the *in vitro* reconstructed intestinal epithelium, EpiIntestinal (Mattek). The tissue integrity was monitored over the course of the experiment using transepithelial electric resistance measurement (TEER). At the end of the dosing period, the cumulative Al^{3+} absorption was assessed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) analysis and the morphological effects were analyzed by standard histological assessment and transmission electron microscopy (TEM). RT-qPCR was used to assess expression of genes associated with oxidative stress.

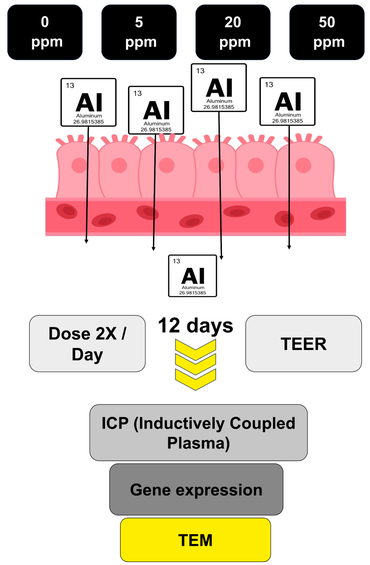

Figure 1: Experimental setup. EpiIntestinal tissues were dosed with Al^{3+} twice a day for 12 days. Barrier integrity was monitored using TEER. At the end of experiment, the cumulative absorption was measured using ICP and the expression of selected genes was analyzed by qPCR. The tissue morphology was assessed using standard H&E staining and by transmission electron microscopy (TEM).

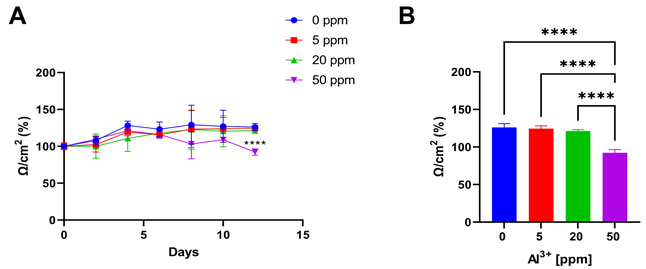

Figure 2: Monitoring of EpiIntestinal barrier integrity using TEER measurements. **A:** TEER over time; **B:** Comparison of average TEER at the termination point. All values represent average measurement of 3 independent tissues normalized to initial TEER, which was considered 100%. Error bars represent standard deviation between measurements.

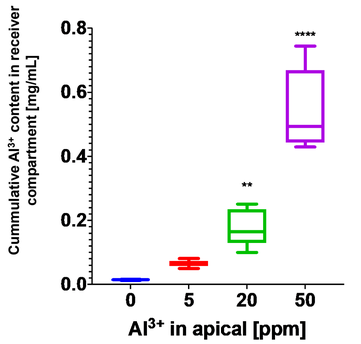

Figure 3: Measurement of cumulative aluminum in receiver fluid after 12 days using inductively coupled plasma-atomic emission spectroscopy.

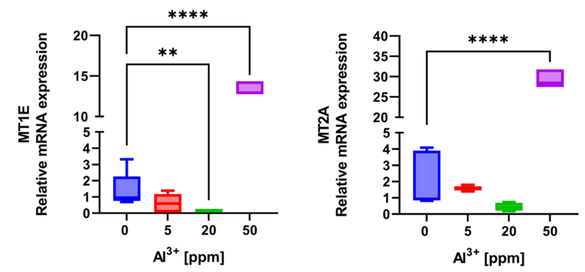

Figure 4: Analysis of gene expression in EpiIntestinal tissues of selected metallothionein isoforms at the experiment termination (day 12). The concentrations of apically applied aluminum are show on x axis. The y axis represents relative expression (fold) change. The data represent the results of two independent experiment. Each qPCR was run N=3.

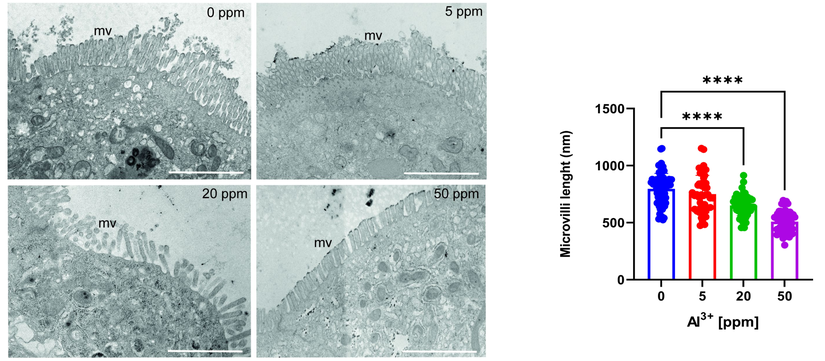

Figure 5: Effects to EpiIntestinal Ultrastructure - Transmission electron microscopy (TEM) analysis of selected tissues. The applied dose is depicted on each figure. The graph summarizes measurement of microvilli length in randomly selected fields. The dots represent measured values and the height of bar represents average length.

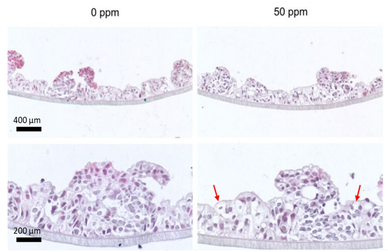

Figure 6: Histology analysis of EpiIntestinal tissues at day 12: left – untreated control, right – tissues treated with 50ppm. Arrows indicate vacuoli formed in the tissues treated with highest concentration

Results

Barrier integrity monitoring via TEER did not reveal any significant differences between tissues exposed to Al^{3+} and controls except for the highest concentration (50 ppm). These findings were consistent with observed expression patterns of genes associated with tight junctions and with level of metallothionein genes associated with different amounts of Al^{3+}. Histological and TEM analyses revealed that the highest Al^{3+} concentration was associated with vacuolization and microvilli shortening, while no pathological changes were observed in other samples.

Discussion and Conclusions

Overall, the results of this study suggest that the repeated exposure to Al^{3+} did not lead to significant detrimental effects of the 3D EpiIntestinal tissue model except for the highest concentration used. These findings contribute to our understanding of the safety of Al^{3+} exposure within the context of the gastrointestinal system since physiological concentrations of aluminum in blood stream are very low, typically < 10 µg/L (0.01 ppm). Nonetheless, further research may be needed to explore any potential long-term effects and to understand the possible implications for human health especially for chronic exposure at high concentrations. The observed morphological changes may deserve further attention.