Rinse Aid Residues in Professional Dishwashers Could Harm Protective Layer of Gut

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Did you know that the skin is the largest organ of the human body, covering a surface area of 22 feet? The skin is the best-known example of an epithelial layer that protects against pathogens and other harmful particles. It is known that tears in the skin due to injury can cause inflammation and activate the associated immunologic reactions. However, have you ever wondered what happens when the epithelial layer lining our gut gets disrupted? According to the "Epithelial barrier hypothesis" , disruption to epithelial barriers in the body is postulated to cause auto-immunogenic and inflammatory diseases, such as systemic lupus erythematosus and inflammatory bowel disease. In accordance, disruption of the intestinal epithelial barrier has been observed in celiac disease and inflammatory bowel disease patients. Numerous substances are known to damage the intestinal epithelium. Among these substances, the harmful effects of dishwashing residues on the intestinal epithelium are less understood and studied.

To uncover this, in the November issue of the *Journal of Allergy and Clinical Immunology*, Ogulur and colleagues employed *in vitro* methodologies and studied the effects of the dishwashing components on cultured cells, organoids, and gut-on-a-chip. An organoid is a self-organized 3D tissue cultured from sources such as stem cells. These stem cells have the capability to differentiate and give rise to a variety of cell types. Organoids exhibit similar functionality to an actual organ and are therefore widely used to study disease pathologies. While the use of organoids does possess benefits in biological research, it is not without drawbacks. Some of the critical limitations of organoids include the absence of mechanical cues and multiscale architecture — such as connective tissues — that the tissues or cells are subjected to *in vivo*. Gut-on-a-chip is an example of an organ-on-a-chip. Organ-on-a-chip is developed to overcome some of the limitations of using organoids.

In essence, organ-on-a-chip is a microfluidic device that contains engineered tissues or cells for the purpose of simulating the function of an organ. Organ-on-a-chip contains chambers that are continuously perfused with fluids such as blood to keep cells viable and subject cells to similar environmental cues that they experience *in vivo*.

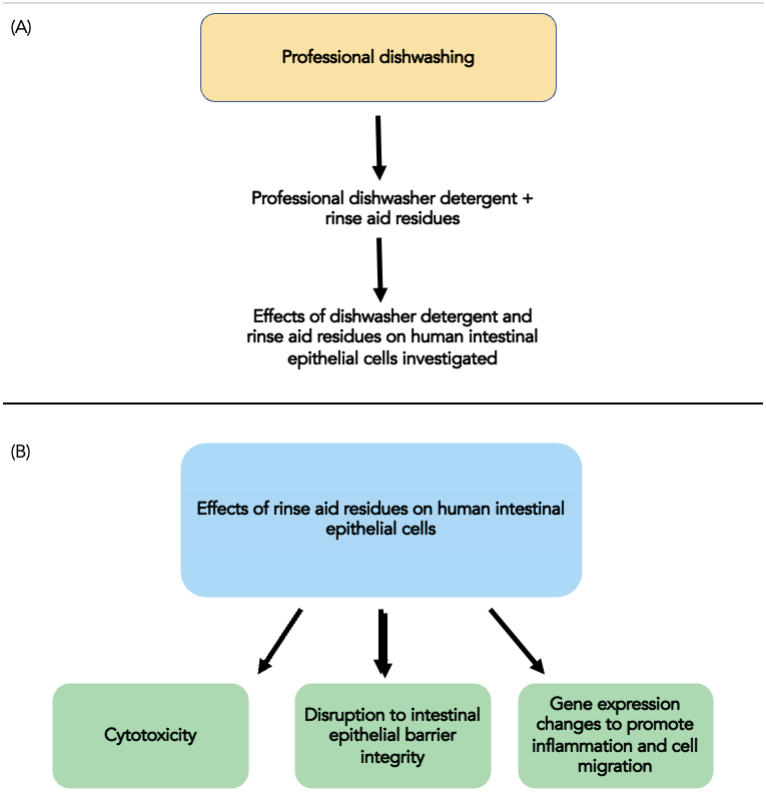
Effective sanitization and high efficiency are two major advantages of professional dishwashers. Nowadays, professional dishwashers are seen in restaurants, hotels, and other places where mass food catering is required. How do professional dishwashers work? In brief, professional dishwashers work by washing dirty dishware with water heated to 65°C and above and dishwashing detergent for 1 minute at pressure varying from 2 to 4 atmospheres. Lastly, the dishware undergoes a rinse and dry cycle. During this process, the plate is rinsed with water. Subsequently, the rinse aid is added and heated to 82°C. It is estimated that the rinse aid residues on the dishware after dishwashing range between 1:2000 – 1:10000 dilutions. For dishwasher detergent residues, it is estimated that the dishwasher detergent residues range from 1:250 - 1:667 dilutions after the dishwashing procedure.

To investigate the effects of dishwasher detergents and rinse aid at these dilutions, cytotoxicity and epithelial barrier integrity were assessed by Ogulur et al. Potential cytotoxic effects of dishwasher residues were analyzed through measurement of cell death and the amount of lactate dehydrogenase (LDH) in the culture medium. LDH is released when the cell is damaged. As such, measurement of the amount of LDH released enables detection of cell damage and cytotoxicity. Measurement of epithelial barrier integrity is assessed using fluorescein isothiocyanate (FITC)-labeled dextran and monitoring changes in transepithelial electrical resistance (TEER). The underlying principle for FITC-labeled dextran is that under normal conditions, cells are well-connected by tight junctions. Many intercellular connections hold cells together. Tight junction proteins are found at the most apical side of cell-to-cell connections. Apical side refers to the side facing the lumen. In this case, the tight junction is similar to cells "holding hands" and therefore forming a barrier to regulate permeability. As such, under normal conditions, dextrans are unable

to pass through the epithelial barrier formed by cells. However, when these tight junctions are disrupted, dextrans will now be able to pass through. This can be visualized by detecting fluorescence signals from FITC-labelled dextrans. For TEER, a decrease in TEER values indicates that the epithelial barrier integrity is compromised.

Contrary to what one would expect, professional rinse aid (1:20000 dilution) but not dishwasher detergent was found to be cytotoxic and responsible for compromising epithelial barrier integrity through disruption to cellular tight junctions. At a concentration of 1:20000 dilution, rinse aid treatment led to significantly increased cytotoxicity of human intestinal epithelial cells. This is further supported by data showing that rinse aid treatment alone exerted cytotoxicity to intestinal epithelial cells at levels comparable to combinatorial treatment of rinse aid and dishwasher detergent. Similar results were obtained from the experiment focusing on intestinal epithelial barrier integrity. Significantly decreased TEER values and increased FITC-dextran passage — implying damaged epithelial barrier — were seen only in rinse aid but not in detergent treatment.

To put to context these results obtained, the authors simulated the process of professional dishwashing on cups and extracted the rinse aid residues that remained. Treatment of intestinal epithelial cells with rinse aids extracted from the simulated professional dishwasher gave the same results, which showed high cytotoxicity and disruption to intestinal epithelial barrier integrity. Notably, these results are obtained despite diluting the extracted rinse aid residues at a dilution factor of 10.



Flowchart and summary of the findings by Ogulur and colleagues. (A) Professional dishwasher detergent and rinse aid residues are left on the dishware after dishwashing. The authors investigated the effects of these professional dishwasher detergent and rinse aid residues on human intestinal epithelial cells. The authors assessed whether professional dishwasher detergent and/or rinse aid residues killed/caused damage to cells, disrupted the integrity of intestinal epithelial barrier and other effects exerted on cells (e.g., changes to what genes or to what extent they are expressed by the cell). (B) Findings by Ogulur and colleagues. Rinse aid residues but not dishwasher detergent was found to cause damage/cell death (cytotoxicity) and disrupted the integrity of the intestinal epithelial barrier. The authors also found that rinse aid residues altered the gene expression of cells to promote inflammation and cell migration.

Claudin-1 (CLDN-1) and occludin (OCLN) are two of the many tight junction proteins that make up the tight junction complex. Alterations in the localization of these tight junction proteins compromise epithelial barrier integrity, leading to the passage of molecules that are otherwise prohibited under normal conditions. Using the technique of immunofluorescence-staining, the team found that professional dishwasher detergent did not affect the expression of CLDN-1 and OCLN at the concentrations studied. Immunofluorescence staining works by using antibodies to bind to targets. These antibodies are conjugated to fluorophores (fluorescence chemical compounds) that can be excited to be visualized. However, opposite observations were seen with rinse aid treatment of intestinal epithelial cells, whereby immunofluorescence staining showed altered localization patterns for CLDN-1 and OCLN. Subsequently, experiments performed on gut-on-a-chip using professional dishwasher detergent and rinse aid also led to the same conclusions.

Naturally, one question comes to mind: which component in rinse aid could have been responsible for the cytotoxicity and damage to the epithelial barrier seen? It is known that rinse aid comprises three components, namely: citric acid, alcohol ethoxylates, and sodium cumenesulfonate. Through testing these components individually and measuring cytotoxicity and the TEER value changes, alcohol ethoxylate was identified as the main component for the harmful effects seen. This is especially clear when alcohol ethoxylate treatment alone led to cytotoxicity and epithelial barrier damage at levels comparable to that when rinse aid is used as a whole to treat intestinal epithelial cells.

Finally, what other effects does rinse aid have apart from cytotoxicity and disruption of tight junctions? To answer this question, the authors analyzed gene and protein expression changes in response to rinse aid treatment. The results suggest that rinse aid treatment significantly elevated molecules regulating proinflammatory responses and cell migration.

What is the significance of this study, and what insights does it provide? In recent decades, there has been considerable growth in the number of patients with allergic and inflammatory diseases. For many of us, the first thought that comes to mind for the cause of allergic and inflammatory diseases would be genetic predispositions. However, given the complexity of biology, it can be appreciated that numerous other factors such as lifestyle could have contributed to the development of allergic and inflammatory diseases. The study by Ogulur and coworkers provide insights and alternative explanations of the possible contributing cause of the increased prevalence of allergic and inflammatory disease over the years. The central idea of Ogulur's work is based on the epithelial barrier hypothesis, which proposes that damage to the epithelial barrier by harmful substances could lead to subsequent inflammation. It is known that excessive activation of inflammatory responses could promote damage to the epithelial barrier and initiate a vicious cycle. The main finding by Ogulur and team is that rinse aid residues after professional dishwashing could have potential cytotoxic and damaging effects on the intestinal epithelial barrier at specific concentrations.

Furthermore, they found that alcohol ethoxylate is the main component of rinse aid contributing to these detrimental effects. These findings highlight the need for a more stringent evaluation of the concentration of alcohol ethoxylate used in rinse aids and prompt the search for safer alternatives to ensure public health safety. As noted by the authors, the present study is limited by the fact that *in vivo* experiments were not conducted. As with all studies, given the intricacy of human biological processes, data obtained from *in vitro* experiments needs to be further confirmed by *in vivo* studies. Nonetheless, the findings by Ogulur and coworkers would help pave the way for uncovering how allergic and inflammatory diseases develop and the reasons for the increased prevalence of these diseases.

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