Effects of emulsifiers on intestinal barrier integrity and exposure to food allergens

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Abstract

Introduction: Enhancement of food allergic responses could be related to cellular membrane effects from certain emulsifiers. Consequently, the interactions among the food matrix components are of importance for risk characterization purposes. **Purpose:** Exploring how emulsifiers (e.g. Polysorbate [P]-80 or Lecithin [LE]) might affect intestinal barrier integrity and transport of allergenic proteins such as the egg allergen ovalbumin (OA). **Methods:** We challenged monolayers of Caco-2 cells, in an *in vitro* model of human epithelial tight junctions with 0.01, 0.05, 0.1, and 0.5% of P-80 or LE alone, and OA alone (0.1, 1, and 10 mg/ml) or in combination (0.5 mg/ml) with emulsifiers (0.2%). We measured toxicity, cellular viability, Lucifer Yellow (LY) penetration, Trans-Epithelial Electrical Resistance (TEER), and expression of tight junction molecules by gene-expression and immunofluorescence assays. An in-house ELISA determined the rate of transport of OA, with or without emulsifiers, to basolateral media. **Results:** All P-80 and OA individual treatments showed minimal effects on cellular viability and toxicity. Concentrations of ≤0.1% LE resulted in cytotoxicity of 0-10% and normal cell proliferation. At 0.5% LE, cytotoxicity increased to 50% with <10% proliferation. When treated with either P-80 or LE at 0.5%, penetration of LY increased significantly; 70-90% over lower concentrations. Although TEER was reduced (~20%) with 0.2% of P-80, using 0.2% LE and 0.5 mg/ml OA had no effect. The expression of tight junction genes and proteins were significantly disrupted in 0.5% LE treated cells compared to 0.2% LE, 0.2% and 0.5% P-80 treated cells. About twice the amount of OA was transported paracellularly in the presence of 0.2% P-80, relative to OA alone or LE treatments suggesting certain emulsifiers can augment allergen absorption process by modulating barrier integrity.

Significance: Our experimental design system has potential to assess the effects of emulsifiers on barrier functions, and how this may secondarily impact allergen absorption from foods.

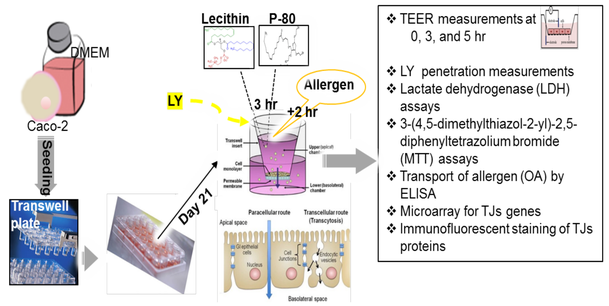
Introduction

- The gate keeping function of the gut epithelium allows efficient passage of nutrients and restricts the entry of harmful antigens. Epithelial tight junction (TJ) integrity can be perturbed by various triggers ranging from environmental (infection, stress), chemicals, larger molecules, and foreign substances. These can enhance allergen absorption, a potentially harmful outcome for individuals who are susceptible to food allergens.
- A major shift of human dietary patterns, such as the increased consumption of processed food associated with a "Western diet", has resulted in the intake of unprocessed or minimally processed foods.
- Industrial practices changed over the past decades providing ultra-processed food to increase productivity and diversity as well as decrease seasonal dependency and prices. Processed foods often contain synthetic or natural ingredients, such as emulsifiers, preservatives, sweeteners, and thickeners.
- Due to unique chemical structures and functions among food ingredients, emulsifiers are used in a range of foods (e.g., bakery, confectionary, dairy, fat and oil, sauces, butter and margarine, ice-cream, cream liqueurs, meat, coffee, gum, beverages, chocolate and convenient food industries).
- Antigens are transported across the gut barrier via different routes, but particularly via trans-cellular or paracellular routes (or both) depending on the health status of the individual.
- A growing number of scientific studies support a possible link between numerous ingredients, including emulsifiers, and the development of various diseases (e.g. celiac disease, food allergy, and inflammatory bowel diseases).
- Emulsifiers can increase TJ permeability through various mechanisms and have often been used to increase the availability of bioactive molecules such as orally administered pharmaceuticals. Since the absorption-enhancing properties of emulsifiers used in pharmaceuticals may potentially increase non-specific absorption, their use in foods should be investigated in relation to allergens.
- Several emulsifiers have been reported to increase paracellular uptake of the egg white allergen ovomucoid, and *in vivo* absorption of soy allergens in individual studies.
- Overall, there is a lack of systematic investigations of the effects of some emulsifiers on paracellular transport across the gut epithelium.

AIM: To understand the relationship between food matrix components, such as emulsifiers, and allergen exposure for risk characterization

- Evaluate the role of some emulsifiers (P-80, and LE) on the intestinal barrier integrity and function.
- Determine the potential impact of altered barrier permeability on the transport of egg allergen OA.

Experimental Design



- TEER measurements at 0, 3, and 5 hr
- LY penetration measurements
- Lactate dehydrogenase (LDH) assays
- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays
- Transport of allergen (OA) by ELISA
- Microarray for TJs genes
- Immunofluorescent staining of TJs proteins

Results

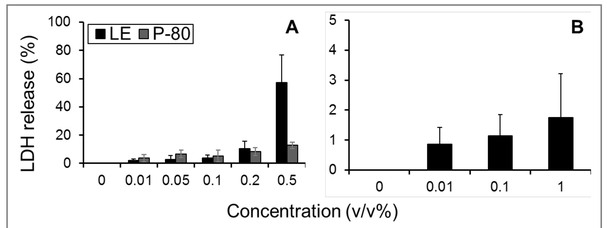


Fig1. Dose-dependent effects of (A) LE, P-80, and (B) OA on cell cytotoxicity determined by LDH assays. Values expressed as means ± SEM, *n* = 3.

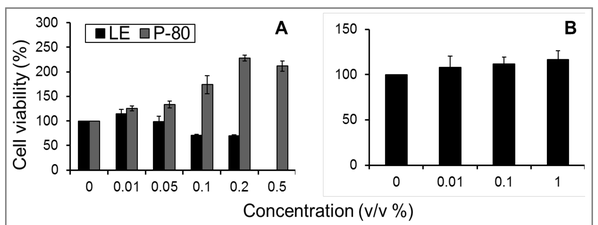


Fig 2. Dose-dependent effects of (A) LE, P-80, and (B) OA on cell viability determined by MTT assays. Values expressed as means ± SEM, *n* = 3.

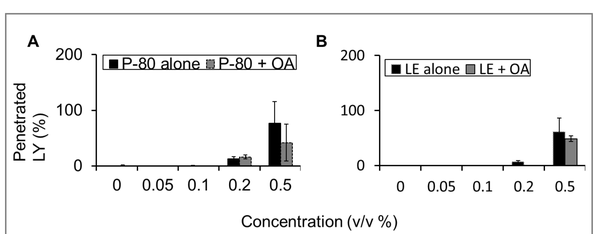


Fig 3. Dose-dependent effects of (A) P-80, and (B) LE on the penetration of LY as an indicator of tight junction integrity of cell monolayers in the presence or absence of OA. Values expressed as means ± SEM, n = 3.

Results

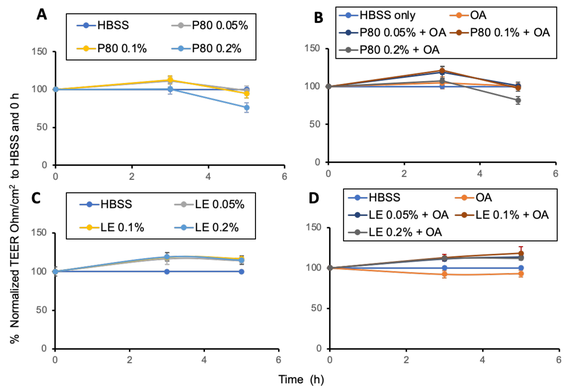


Fig 4. Relative changes in TEER of cell monolayers after exposure to incremental concentrations of (A) P-80 or (C) LE, and (B,D) in combination with OA. Data reported as the percentage normalized with high salt buffered saline (HBSS) control, then with initial time point (t=0) of each treatment. Values expressed as means ± SEM, *n* = 3.

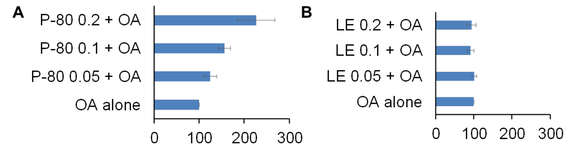


Fig 5. Dose-dependent effects of (A) P-80, and (B) LE on penetrated quantity of allergens after indicated treatments for 5h. Data represents three independent experiments in triplicate, in which the penetrated OA were quantified by ELISA in triplicates and reported as the percentage, relative to allergens alone. Values expressed as means ± SEM, n = 27.

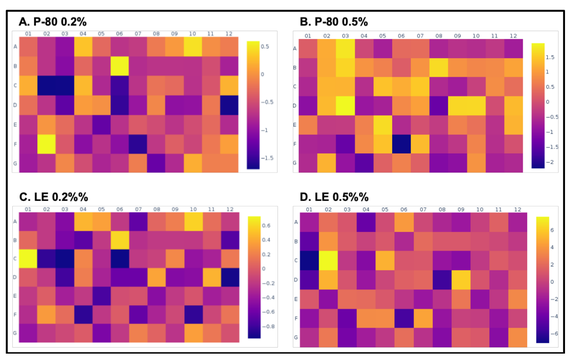


Figure 6. Visualization of Log_2 fold change of mRNA expression of TJ-associated genes induced by 0.2% of P-80 (A) , 0.5% of P-80 (B), 0.2% of LE (C), and 0.5% of LE (D) treatments of cell monolayers for 5h.

FDA Mission Relevance: This study provides relevant and predictive *in vitro* data for human safety assessments of chemicals of Agency interest and an accurate base of information for making relevant regulatory decisions.

Table 1. Log_2 fold change of mRNA expression of indicated TJ-associated genes of cell monolayers treated with P-80 and LE for 5h.

Human tight junction associated genes	Fold regulation			
	P-80 0.2%	P-80 0.5%	LE 0.2%	LE 0.5%
CLDN1 (Claudin 1)	-1.11	-1.28	1.05	-22.5
CLDN4 (Claudin 4)	1.13	1.66	-1.48	-137.09
CLDN5 (Claudin 5)	-3.25	2.35	-1.8	185.02
OCLN (Occludin)	-1.34	-2.65	1.07	-16.98
TJP1 (Tight junction protein 1 (zona occludens 1))	-2.38	-2.14	1.03	-8.72

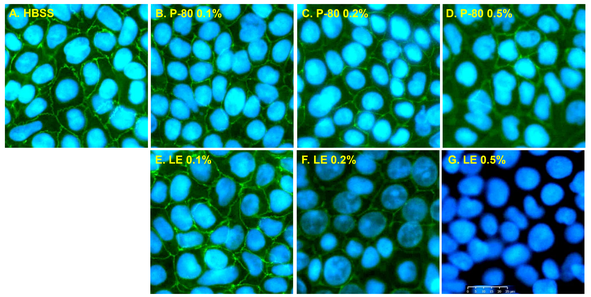


Fig 7. Expression of OCLN proteins (green fluorescence) in HBSS control (A); 0.1%, 0.2% and 0.5% P-80 (B-D) and LE (E-G) treated cell monolayers for 5h. Cell nuclei were counterstained with DAPI.

Conclusion

- No significant release of LDH (≤ 10%) occurred at lower concentrations (0.01, 0.05, 0.1 and 0.2%; V/V) of P-80 and LE (Figure 1A). But remarkable differences occurred as the concentration increased to 0.5%. Treatment of cells with OA, at indicated concentrations, showed little (≤ 5%) to no effect (Figure 1B).
- P-80 seemed to induce cell proliferation in a dose dependent manner, while a decrease occurred, with increased LE concentrations (Figure 2A). Results revealed that OA did not alter the viability of the cells at all treatment concentrations (Figure 2B).
- P-80 alone or in combination with OA treatments resulted in significant increase in penetrated LY ($p < 0.05$) at less cytotoxic concentration of 0.2%. The effect was mainly attributed to P-80 (Figure 3A); conversely LE showed less effect at 0.2% concentration (Figure 3B).
- Treatments with OA alone did not interfere with cell monolayers (Figure 4B; 4D). Only P-80 reduced TEER about 20% in a concentration- dependent manner (Figure 4B). LE had no effect on TEER at all tested concentrations (Figure 4A). P-80 in combinations with OA (Figure 4A) affected the TEER in the same manner and magnitude as observed with P-80 alone. Similar to the effect of LE alone treatments, combinations with OA (Figure 4D) did not show any effect on TEER.
- The permeated allergens across cell monolayers treated with OA alone indicated absorption via a transcellular route, because allergens alone neither decreased TEER nor increased permeated LY. The transport rate of OA (Figure 5A) markedly increased in the presence of P-80 and was dependent on the concentration. LE was unable to alter the transport rate of OA (Figure 5B).
- Focusing on predominant claudins (CLDN1, CLDN4 and CLDN5), OCLN, and zonula occludens, crucial for the maintenance of epithelial barrier integrity, we observed a reduction in their expression in 0.2% P-80 treated cells compared to mostly unchanged expression in 0.2% LE treated cells (Table 1). Overall, a slight decrease was found in P-80 (0.2 and 0.5%) treated cells, but the effect of LE was significant in a concentration-dependent manner.
- Both P-80 and LE treatments decreased immunostaining for OCLN in a concentration-dependent manner. However, the staining intensities and distributions were comparable to control up to 0.2% treatments of both emulsifiers and the changes were significant when the concentrations increased to 0.5% (Figure 7).

Overall, our research model has the potential to analyze food ingredients affecting paracellular permeability for biological safety. However, this model may not completely address the complexity of an *in vivo* situation.