How do haloacetamides and haloacetic acids affect human intestinal epithelial cells?

Karlien Dejaeger, Justine Criquet, Marjolein Vanoppen, Emile R. Cornelissen, Gabriel Billon, Cécile Vignal

3rd IWA Specialized International Conference on Disinfection and DBPs 2022
June 27th – July 1st, 2022 - Milan, Italy

How do haloacetamides and haloacetic acids affect human intestinal epithelial cells?

K. Dejaeger[1,2,3], J. Criquet[2], M. Vanoppen[1,4], E.R. Cornelissen[1,4,5], G. Billon[2] and C. Vignal[3]

[1] PaInT, Particle and Interfacial Technology group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[2] Univ. Lille, CNRS, UMR 8516 - LASIRE, Laboratoire Avancé de Spectroscopie pour les Interactions, la Réactivité et l'Environnement F-59000 Lille, France
[3] Univ. Lille, Inserm, CHU Lille, U1286 - INFINITE - Institute for Translational Research in Inflammation, F-59000 Lille, France
[4] Centre for Advanced Process Technology for Urban Resource Recovery (CAPTURE), www.capture-resources.be, Belgium
[5] KWR Water Research Institute, Groningenhaven 7, 3433 PE Nieuwegein, Netherlands

* Corresponding author: karlien.dejaeger@ugent.be

Summary: In this research, human intestinal epithelial cells (Caco-2) were exposed to haloacetamides and haloacetic acids. A 24, 48 and 72h exposure was conducted to determine the cytotoxicity of these compounds. The unregulated haloacetamides were clearly more cytotoxic than the regulated haloacetic acids. Moreover, changing the halogen from Cl to I increased the toxicity extensively. A 6h stimulation with 1 µM of each compound and subsequent RNA extraction showed high upregulation of genes involved in both oxidative stress and inflammatory pathways with haloacetamide exposure, while no significant changes were seen for haloacetic acid exposure.

Keywords: cytotoxicity; gene expression; Caco-2

INTRODUCTION

Disinfection of drinking water is a widely used technique to inactivate pathogens. However, harmful disinfection by-products (DBPs) are formed due to the reaction with organic matter (Pals et al., 2013). Unregulated DBPs, such as haloacetamides (HAcAms), are found in lower concentrations compared to the regulated trihalomethanes and haloacetic acids (HAAs), but it is suggested by several studies that their toxicity is much higher (Richardson et al., 2007).

Research with Chinese Hamster Ovary (CHO) cells showed that HAcAms are about 140 times more cytotoxic and 10 times more genotoxic than the regulated HAAs (Ding et al., 2019). Only very few research has been conducted on human cells, although the CHO results cannot necessarily be extended to human cell lines (Ding et al., 2019). For example, studies on colonic epithelial cells (CCD 841 CoN) showed that iodoacetic acid is 4 times more cytotoxic than iodoacetamide, whereas iodoacetamide has 2 times higher cytotoxicity than iodoacetic acid in CHO cells (Sayess et al., 2017; Wagner and Plewa, 2017).

Furthermore, reactive oxygen species and the activation of oxidative stress pathways are at the basis for the genotoxic and carcinogenic properties of the DBPs. Nonetheless, limited studies have investigated inflammatory responses despite the well-established relationship between these responses and the progression of cancer (Procházka et al., 2019).

This research will therefore, (i) compare the cytotoxicity between HAAs and HAcAms and (ii) investigate the expression of various genes for both families on human intestinal epithelial cells (Caco-2).

MATERIALS AND METHODS

Caco-2 cells were grown at 37°C under a 5% CO_2 humidified atmosphere in DMEM medium supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin, 2mM L-glutamine and 20% fetal calf serum.

10^4 cells/well were seeded in a 96-well plate and incubated with 100 µL cell medium containing increasing concentrations of either chloroacetamide (Cl-AcAm), bromoacetamide (Br-AcAm), iodoacetamide (I-AcAm), dichloroacetamide (Cl_2-AcAm), trichloroacetamide (Cl_3-AcAm), chloroacetic acid (Cl-AA), bromoacetic acid (Br-AA) or iodoacetic acid (I-AA) for 24h, 48h and 72h. 10 µL of 5 mg/mL MTT (3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide) stock solution was added at the end of the stimulation period and incubated for 4h at 37°C in a 5% CO_2 humidified atmosphere. The MTT is reduced by metabolic active cells and converted into formazan crystals. These crystals were dissolved with 100 µL DMSO and absorbance was measured at 570 nm. The results were used to plot dose-response curves.

$5x10^5$ cells/well were seeded in a 24-well plate and incubated with 300 µL cell medium containing a 1µM concentration of a certain compound for 6h. RNA was extracted using the Nucleospin RNA II kit (Macherey Nagel) and subsequently converted into cDNA. qPCR was conducted for the detection of genes involved in inflammation and oxidative stress pathways.

RESULTS AND DISCUSSION

Figure 1 shows the half maximal inhibitory concentration (IC_{50}) deduced from the dose-response curves ($r^2 > 0.75$) for HAcAms and HAAs. From this, it is concluded that for both families, the I-DBP is the most cytotoxic followed by Br-DBP. Cl-DBP appears to be the least cytotoxic. This is in agreement with studies exerted on CHO cells (Plewa et al., 2010; Plewa et al., 2008). Cl_2-AcAm and Cl_3-AcAm caused least cell death, with IC_{50} values up to 40 000 µM.

Furthermore, the HAcAms have overall lower IC_{50} values compared to the HAAs, which is similar to the conclusions drawn from CHO cells (Wagner and Plewa, 2017). However, the IC_{50} values found in this study were generally higher than the ones reported for CHO (Wagner and Plewa, 2017).

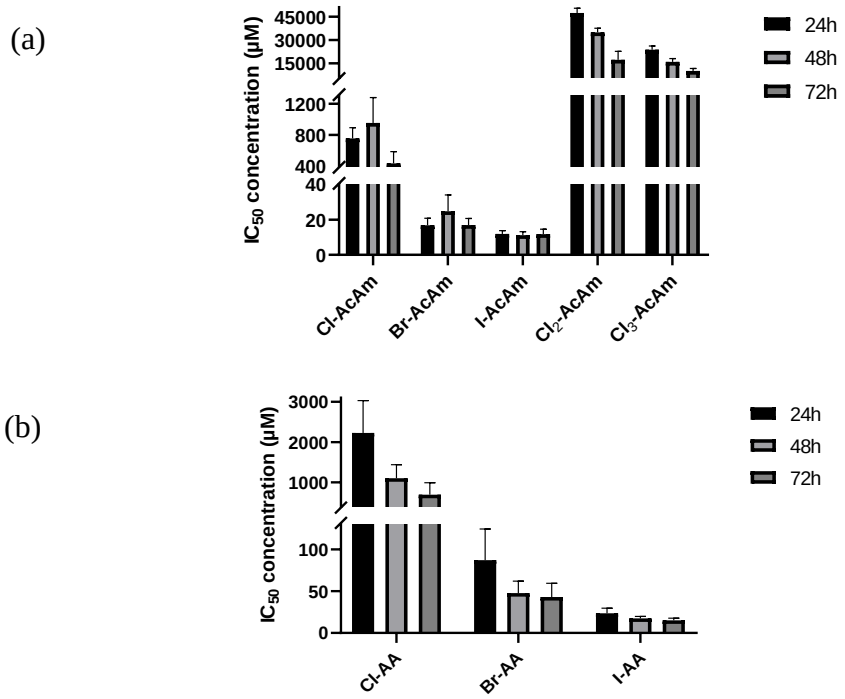

Figure 1: IC_{50} values (µM) determined after 24h, 48h and 72h for, (a) chloroacetamide (Cl-AcAm), bromoacetamide (Br-AcAm), iodoacetamide (I-AcAm), dichloroacetamide (Cl_2-AcAm), trichloroacetamide (Cl_3-AcAm) and, (b) chloroacetic acid (Cl-AA), bromoacetic acid (Br-AA) and iodoacetic acid (I-AA). Error bars represent the 95% confidence interval.

A preliminary assessment on gene expression for inflammation and oxidative stress pathways with Caco-2 cells was exerted and is shown in Figure 2. The 1 µM dose was chosen, since this concentration appeared to be non-cytotoxic for all compounds after 24h.

Figure 2a shows a prominent change in gene expression from Cl-AcAm to I-AcAm. No change in gene regulation was seen for Cl-AcAm, but Br-AcAm shows an upregulation for oxidative stress genes. On the other hand, I-AcAm shows high upregulation for all inflammatory genes tested. Although Cl_2-AcAm and Cl_3-AcAm did not show high cytotoxicity, they did have an effect on several genes. No significant changes were seen for HAAs (Figure 2b)

Although it is believed that DBPs are toxic through the activation of oxidative stress pathways, this preliminary study shows high expression in inflammatory pathways. This confirms previous work performed on FHs74Int cells after a 4h stimulation and subsequent global gene microarray analysis (Procházka et al., 2019).

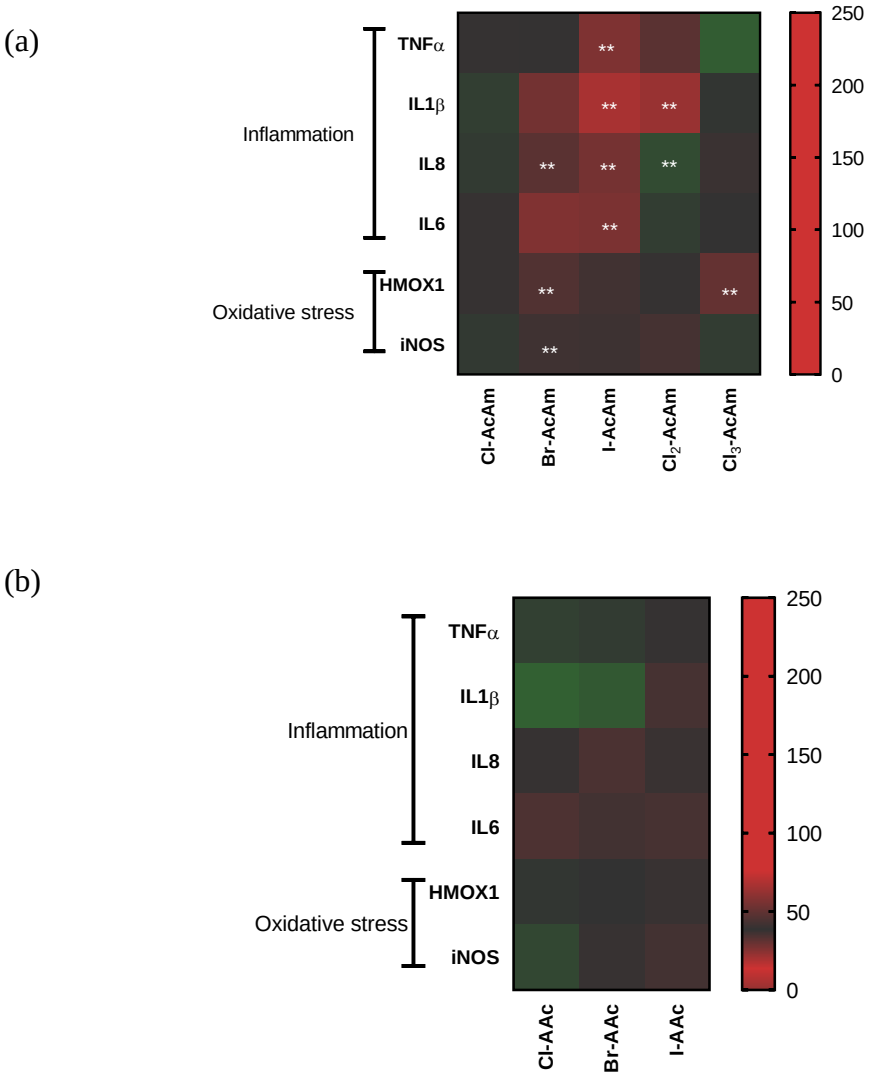

Figure 2: Heatmap showing the expression of inflammatory genes (TNFα, IL1β, IL8, IL6) and oxidative stress genes (HMOX1, iNOS) in (a) chloroacetamide (Cl-AcAm), bromoacetamide (Br-AcAm), iodoacetamide (I-AcAm), dichloroacetamide (Cl_2-AcAm), trichloroacetamide (Cl_3-AcAm) and, (b) chloroacetic acid (Cl-AA), bromoacetic acid (Br-AA) and iodoacetic acid (I-AA). Upregulation compared to the control group is visualized with red (>100%), downregulation with green (<100%). **=Significant different compared to the control group.

The following step in this research will be performing a global gene microarray on human intestinal epithelial cells. This will yield information about the entire set of genes expressed during the exposure of different DBPs and thereby identifying the several pathways that are affected by DBP exposure (Procházka et al., 2019).

REFERENCES

Ding, X., Zhu, J., Wang, X., Zhou, W., Wu, K., Zhou, Z., Zhou, K., Wu, D., Jiao, J., Xia, Y. and Wang, X. 2019. Different cytotoxicity of disinfection by-product haloacetamides on two exposure pathway-related cell lines: Human gastric epithelial cell line GES-1 and immortalized human keratinocyte cell line HaCaT. Science of The Total Environment 692, 1267-1275.

Pals, J., Attene-Ramos, M.S., Xia, M., Wagner, E.D. and Plewa, M.J. 2013. Human Cell Toxicogenomic Analysis Linking Reactive Oxygen Species to the Toxicity of Monohaloacetic Acid Drinking Water Disinfection Byproducts. Environmental Science & Technology 47(21), 12514-12523.

Plewa, M., Simmons, J., Richardson, S. and Wagner, E. 2010. Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. Environmental and molecular mutagenesis 51, 871-878.

Plewa, M.J., Muellner, M.G., Richardson, S.D., Fasano, F., Buettner, K.M., Woo, Y.-T., McKague, A.B. and Wagner, E.D. 2008. Occurrence, Synthesis, and Mammalian Cell Cytotoxicity and Genotoxicity of Haloacetamides: An Emerging Class of Nitrogenous Drinking Water Disinfection Byproducts. Environmental Science & Technology 42(3), 955-961.

Procházka, E., Melvin, S.D., Escher, B.I., Plewa, M.J. and Leusch, F.D.L. 2019. Global transcriptional analysis of nontransformed human intestinal epithelial cells (FHs 74 Int)after exposureto selected drinking water disinfection by-products. Environmental Health Perspectives 127(11).

Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R. and DeMarini, D.M. 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutation Research-Reviews in Mutation Research 636(1-3), 178-242.

Sayess, R., Khalil, A., Shah, M., Reckhow, D.A. and Godri Pollitt, K.J. 2017. Comparative Cytotoxicity of Six Iodinated Disinfection Byproducts on Nontransformed Epithelial Human Colon Cells. Environmental Science & Technology Letters 4(4), 143-148.

Wagner, E.D. and Plewa, M.J. 2017. CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: An updated review. Journal of Environmental Sciences 58, 64-76.