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

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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 μ M 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% CO₂ 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 μ L cell medium containing increasing concentrations of either chloroacetamide (Cl-AcAm), bromoacetamide (Br-AcAm), iodoacetamide (I-AcAm), dichloroacetamide (Cl₂-AcAm), trichloroacetamide (Cl₃-AcAm), chloroacetic acid (Cl-AA), bromoacetic acid (Br-AA) or iodoacetic acid (I-AA) for 24h, 48h and 72h. 10 μ L 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% CO₂ humidified atmosphere. The MTT is reduced by metabolic active cells and converted into formazan crystals. These crystals were dissolved with 100 μ L DMSO and absorbance was measured at 570 nm. The results were used to plot dose-response curves.

5×10^5 cells/well were seeded in a 24-well plate and incubated with 300 μ L cell medium containing a 1 μ M 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 (IC₅₀) 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). Cl₂-AcAm and Cl₃-AcAm caused least cell death, with IC₅₀ values up to 40 000 μ M.

Furthermore, the HAcAms have overall lower IC₅₀ values compared to the HAAs, which is similar to the conclusions drawn from CHO cells (Wagner and Plewa, 2017). However, the IC₅₀ values found in this study were generally higher than the ones reported for CHO (Wagner and Plewa, 2017).

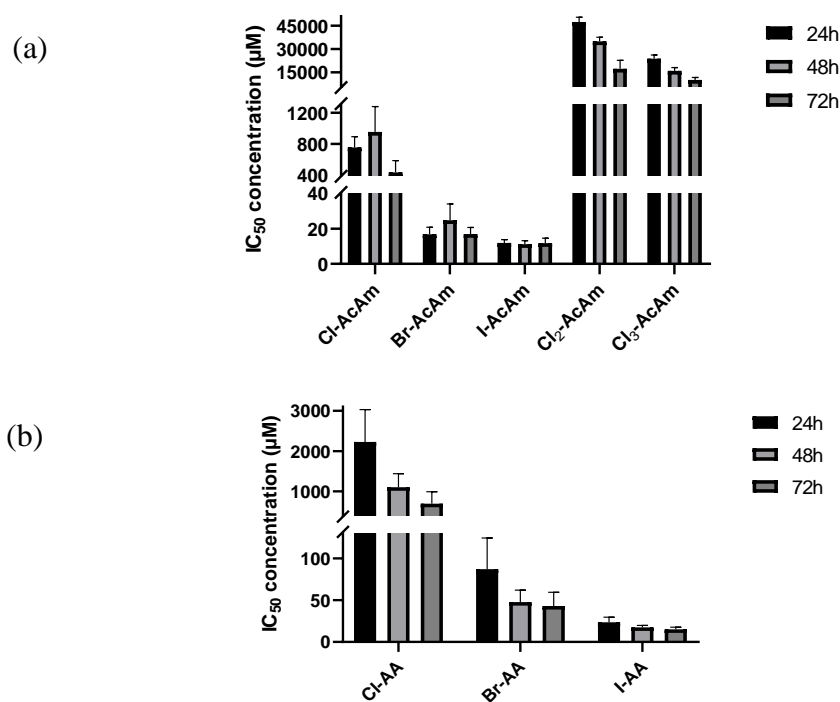


Figure 1: IC₅₀ values (μ M) determined after 24h, 48h and 72h for, (a) chloroacetamide (Cl-AcAm), bromoacetamide (Br-AcAm), iodoacetamide (I-AcAm), dichloroacetamide (Cl₂-AcAm), trichloroacetamide (Cl₃-AcAm) and, (b) chloroacetic acid (Cl-AA), bromoacetic acid (Br-AA) and iodoacetic acid (I-AA). Error 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 μ M 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 Cl₂-AcAm and Cl₃-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).

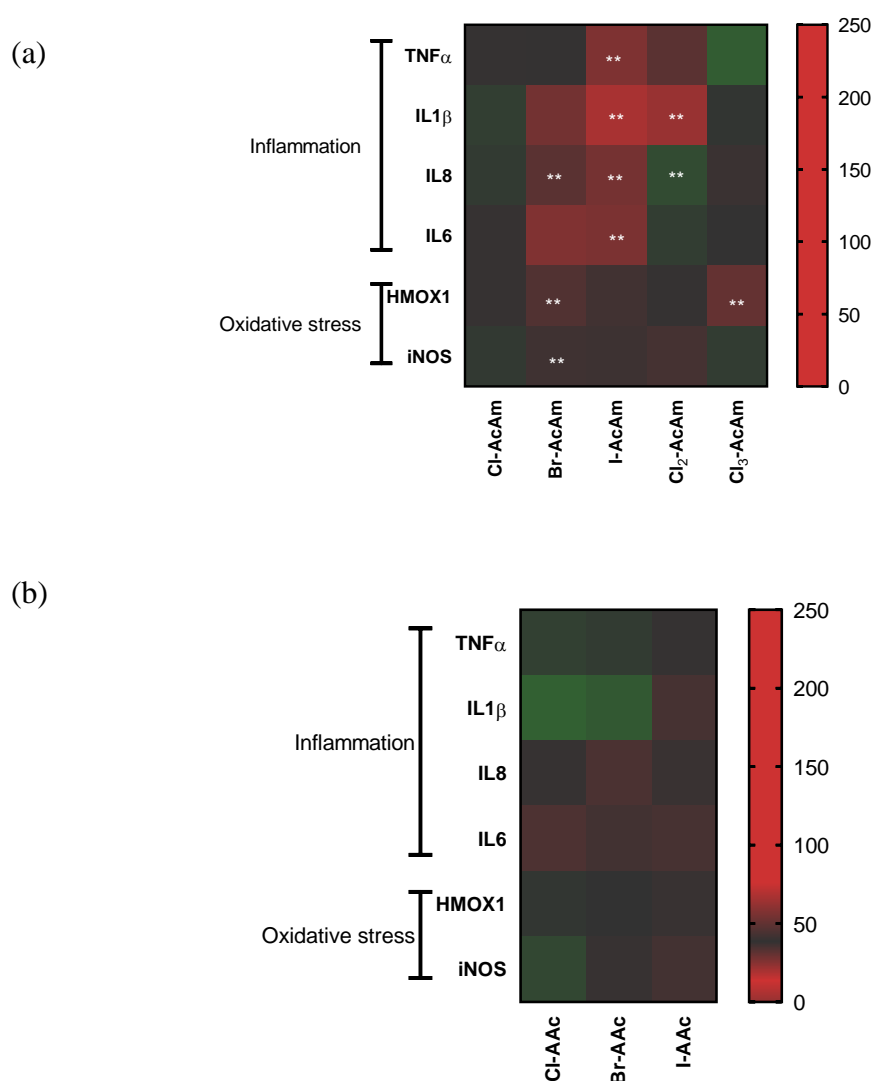


Figure 2: Heatmap showing the expression of inflammatory genes (TNF α , IL1 β , IL8, IL6) and oxidative stress genes (HMOX1, iNOS) in (a) chloroacetamide (Cl-AcAm), bromoacetamide (Br-AcAm), iodoacetamide (I-AcAm), dichloroacetamide (Cl₂-AcAm), trichloroacetamide (Cl₃-AcAm) and, (b) chloroacetic acid (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).

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