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CHLOROACETALDEHYDE, AND NOT ACROLEIN, IS THE MORE URO-TOXIC METABOLITE OF CYCLOPHOSPHAMIDE AND ISOFOSFAMIDE IN VITRO.

Hypothesis / aims of study: Cyclophosphamide (CPO) and ifosfamide (IFO) are commonly used anticancer and immunosuppressive agents. A major limiting factor in the use of CPO and IFO is the resulting uro-toxicity thought to be caused, in part, by reactive oxygen species formation and resulting in ongoing bladder pain, urgency and dysuria. The uro-toxicity of these drugs has been largely attributed to the formation of the metabolite acrolein. However another toxic metabolite chloroacetaldehyde, has been implicated in the neuro-, cardio- and nephro-toxicity of these drugs, but possible uro-toxicity has not been well investigated.
These drugs and their metabolites are excreted in the urine and come into contact with the urothelium. The urinary concentration of acrolein or chloroacetaldehyde likely to occur in patients is 100nM or 10uM respectively (1-3). It is well known that the urothelium plays an important role in maintaining normal bladder function, releasing a number of mediators (eg. ATP and acetylcholine) that can influence sensory nerve sensitivity and detrusor muscle contraction. Accordingly, this study aimed to investigate the affects of the CPO and IFO metabolites acrolein and chloroacetaldehyde on human urothelial cell viability and function in vitro.

Study design, materials and methods: Human urothelial cells (RT4 and T24 lines) were treated with acrolein (10nM – 100µM) or chloroacetaldehyde (1nM – 10µM) for 24 hours. Following treatment, changes in cell viability (resazurin reduction assay) and reactive oxygen species formation (DCFH-DA fluorimetry) were measured. Samples were also prepared for analysis of basal and stimulated ATP, acetylcholine and prostaglandin E_2 (PGE_2) release by incubating cultures in normal (280 mOsm/L) or hypotonic (180 mOsm/L) Krebs solution (which mimics cell stretch) respectively for 15 minutes.

Results:
Twenty-four hour treatment with acrolein (10µM and 100µM) or chloroacetaldehyde (1µM and 10µM) significantly decreased cell viability in both RT4 and T24 cells lines (Fig 1). The decrease in cell viability was accompanied by a significant increase in reactive oxygen species formation after 24 hour acrolein treatment at 100µM in RT4 cells and at 10µM in T24 cells and by 10µM chloroacetaldehyde treatment in both cell lines.

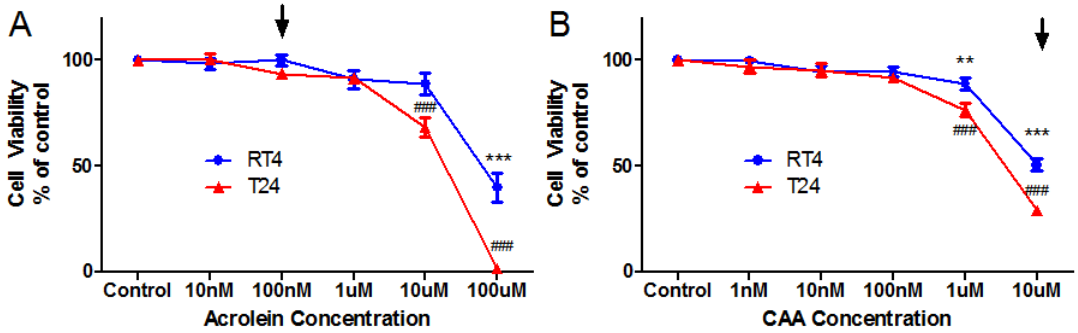

*Fig 1: Effect of 24 hour acrolein (A) and chloroacetaldehyde (CAA) (B) treatment on cell viability of RT4 and T24 cells. Arrows indicate peak urinary concentration in patients. Data are shown as a percentage of control (mean ± SEM, n=6) and analysed by 1-way ANOVA with Dunnett posttest (*** p<0.001, ** p<0.01).*

ATP, acetylcholine and PGE_2 were undetectable in samples from T24 cells treated with 100µM acrolein as cell viability was too low and no other concentration investigated had a significant effect on these mediator levels. However in the RT4 cells treatment with 100µM acrolein caused a 5-fold increase in basal ATP levels ($p<0.001$), 2.5-fold increase in stimulated ATP levels ($p<0.05$) and a 60% increase in stimulated acetylcholine levels ($p<0.001$). PGE_2 levels in basal and stimulated samples from RT4 cells were also increased after treatment with 100µM acrolein (7.5- and 4-fold respectively, $p<0.001$).

In the T24 cell line (but not the RT4 cell line), chloroacetaldehyde (10µM) caused a significant increase in basal and stimulated ATP levels (3.5-fold increase in basal samples and 5-fold increase in stimulated samples, $p<0.001$) (Fig 2-A). In the T24 cells only, basal levels of acetylcholine were significantly reduced while stimulated levels were significantly increased after treatment with 10µM chloroacetaldehyde (50% decrease in basal levels $p<0.05$ and 2.5-fold increase in stimulated levels $p<0.01$) (Fig 2-B). In both cell lines, basal PGE_2 levels were increased significantly (approximately 2.5-fold increase in both cell lines, $p<0.05$) with stimulated levels showing a non-significant increase after chloroacetaldehyde treatment (10µM) (Fig 2-C).

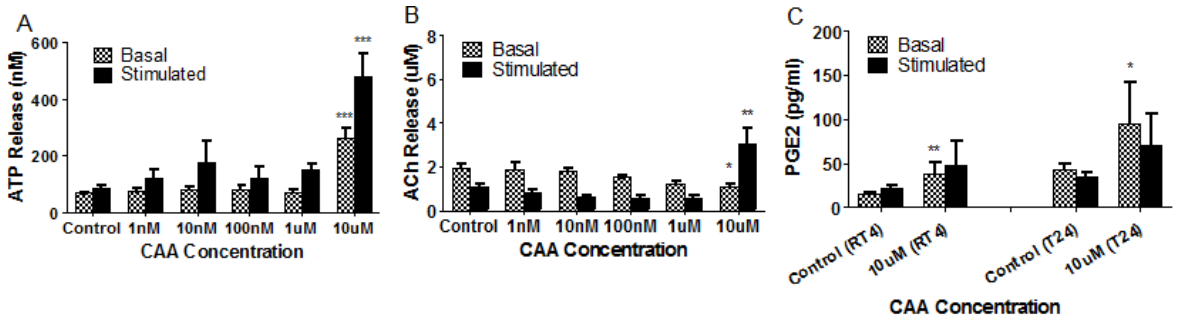

Fig 2: Effect of 24 hour chloroacetaldehyde (CAA) treatment on basal and hypo-osmotically stimulated ATP (A) and acetylcholine (B) levels from T24 cells and PGE_2 (C) levels from RT4 and T24 cells. Data are shown as mean ± SEM (n=6) and analysed using 1-way ANOVA with Dunnett posttest or Students t-test. (p<0.05, ** p<0.01, *** p<0.001, treated v control).*

Interpretation of results: Stretch of the urothelium during bladder filling is known to stimulate the release of ATP which acts on low threshold Aδ sensory nerve fibres in the suburothelium to initiate the micturition reflex. At high concentrations it may act on high threshold nerve fibres to give rise to perceptions of pain. The role of acetylcholine release from the urothelium is unclear with some suggesting it can act in an autocrine fashion to stimulate release of ATP, nitric oxide and PGE_2. It has also been suggested that acetylcholine can affect afferent nerve activity with both inhibitory and stimulatory effects being reported, while PGE_2 is known to sensitise afferent nerves.
At urinary concentrations reached in patients, acrolein had no effect on mediator release from either RT4 or T24 urothelial cells. Whereas chloroacetaldehyde (10µM) enhanced both basal and stimulated levels of ATP from the T24 urothelial cells at a concentration likely to be found in the urine of patients, which may explain the pain and urgency experienced by patients after treatment with cyclophosphamide and ifosfamide. Basal PGE_2 levels were also increased at this concentration which may lead to sensitisation of the afferent nerves to ATP, contributing to the increased sensations of pain and urgency. Changes in basal and stimulated acetylcholine levels were also observed after 10µM chloroacetaldehyde treatment which through autocrine activity may partly contribute to the increased ATP and PGE_2 stimulated release or may even directly affect the afferent nerves.

Concluding message: At urinary concentrations reached in patients, acrolein had no effect on urothelial cell viability, ROS formation or release of ATP, acetylcholine or PGE2. However, chloroacetaldehyde at a concentration likely to occur in the urine of patients (10µM) altered urothelial cell viability, reactive oxygen species production and affected the release of urothelial mediators. The changes observed after chloroacetaldehyde treatment, in particular the large increase in basal and stimulated ATP levels as well as the increased PGE_2 levels, may contribute to the bladder pain, urgency and dysuria seen after cyclophosphamide or ifosfamide treatment. These results suggest that chloroacetaldehyde, not just acrolein, may play a part in the urotoxicity of CPO and IFO and that chloroacetaldehyde may even be more uro-toxic at urinary concentrations reached in patients.

References

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Disclosures

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