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# ACROLEIN, A METABOLITE OF CYCLOPHOSPHAMIDE ENHANCES BASAL ATP RELEASE AND REACTIVE OXYGEN SPECIES FORMATION IN CULTURED HUMAN UROTHELIAL CELLS

Hypothesis / aims of study

Cyclophosphamide is a commonly used anticancer and immunosuppressive agent. A major limiting factor in the use of cyclophosphamide is the resulting bladder toxicity thought to be caused, in part, by reactive oxygen species formation and resulting in ongoing bladder pain, urgency and dysuria. 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. This study investigates the affects of cyclophosphamide and its toxic metabolite, acrolein, on human urothelial cell viability and function in vitro.

Study design, materials and methods

Human urothelial cells (RT4) were treated with cyclophosphamide (0.01 – 100uM) or acrolein (0.01 - 100nM) for 24 hours. Following treatment, effects were measured in terms of changes in cell viability (resazurin reduction assay) and reactive oxygen species formation (DCFH-DA fluorimetry). Samples of incubation media were also prepared for analysis of basal and stimulated ATP and acetylcholine release by incubating cultures in normal (280 mOsm/L) or hypotonic (180 mOsm/L) Krebs solution (which mimics cell stretch) respectively for 15 minutes. The level of ATP in these samples was then measured using a Luciferin/Luciferase assay while the concentration of acetylcholine was measured using an Amplex Red Acetylcholiine Assay Kit.

Results

Twenty-four hour cyclophosphamide treatment did not affect cell viability even at the highest concentration of 100µM, whereas treatment with acrolein for the same duration resulted in a significant decrease in cell viability at a relatively low concentration of 100nM. Similarly, reactive oxygen species formation was not affected by 100µM cyclophosphamide treatment, but was 3-fold higher (P<0.05) in cells treated with 100nM acrolein.

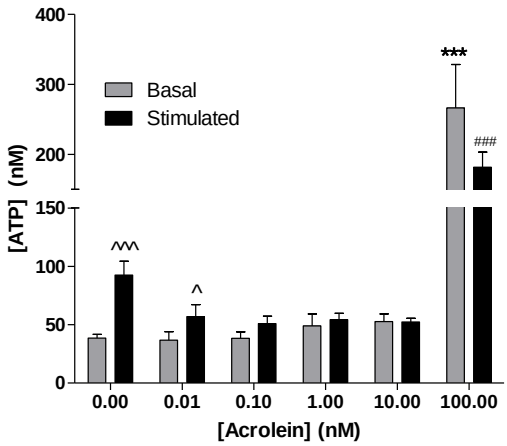


***Figure 1: Effect of 24 hour acrolein treatment on basal and stimulated release of ATP from human urothelial cells. * P<0.001 compared to control basal, # P<0.001 compared to control stimulated, ^ P<0.05 and ^^ P<0.01 compared to basal***

Basal and stimulated acetylcholine release was not altered by acrolein treatment at any of the concentrations examined, but it did have effects on ATP release and these were concentration related (Figure 1). At low concentrations (0.01 – 10nM), basal ATP levels were unchanged but release induced by hypotonic stimulation (ie. cell stretch) was reduced (0.01nM) or abolished (0.1 – 10nM). In contrast treatment with acrolein at a concentration of 100nM, resulted in a 7-fold increase in basal ATP and a 2-fold increase in stimulated ATP levels compared to controls. With this very high basal ATP release after treatment with 100nM acrolein, the response to hypotonic stimulation above basal was abolished (Figure 1).

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. At 100nM, a concentration within the range observed in patients, acrolein enhanced ATP release from urothelial cells and explain the pain and urgency experienced by patients. Given the significant increase in ROS formation observed at the same concentration it is possible that ROS plays a causal role in the alterations in ATP observed following exposure to acrolein.

Concluding message

The cyclophosphamide metabolite, acrolein alters urothelial cell viability and reactive oxygen species production at relatively low concentrations (100nM), while the parent drug, at concentrations up to 100µM, does not. Exposure to acrolein also causes a large increase in the basal release of ATP from urothelial cells and this may contribute to the bladder pain, urgency and dysuria seen after cyclophosphamide treatment.

Disclosures
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