Ultra-high concentrations of gaseous nitric oxide show rapid cytotoxic capabilities against colon, breast, pancreatic and other cancer cells in vitro

Hila Confino1, Matan Goldshtein1, Shani Puyesky1, Shay Yarkoni1, Amir Avniel1, Steve Liss1, Ido Wolf1
1. Beyond Cancer, Rehovot, Israel. 2. Beyond Air Ltd., Rehovot, Israel. 3. Beyond Air Inc., Garden City, NY, USA. 4. Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.

Introduction

- High concentrations of Nitric Oxide (NO) are known to have anti-tumor effects, including cancer cell cytotoxicity through DNA damage induction, leading to apoptotic cell death.
- To examine the cytotoxic effect of gaseous NO (gNO) on various cancer types, we exposed 6 cancer cell lines to gNO at ultra-high concentrations, of 10,000-100,000 ppm for up to 10 minutes.
- Cell viability was measured 24 hours after exposure by (figure 1):
  * XTT-based cell proliferation method
  * Colony-forming assay (Clonogenic assay) – measures the ability of a single cell to grow into a colony

Results – Short exposure of ultra high concentration gNO limits cell viability in human pancreatic and ovarian cancer cell lines

- Human ovarian (figure 2A) and pancreatic (figure 2B) cancer cell lines were exposed to gaseous nitric oxide at 10,000 ppm - 100,000 ppm NO for 10 seconds to 10 minutes. Cell viability was assessed using XTT assay. Less than 10% of both cell lines are viable after 1 minute of exposure to 25,000 ppm NO.

Conclusions

- Exposure of human ovarian and pancreatic cancer cell lines and mouse lung, melanoma, colon and breast cancer cell lines to 10,000 ppm – 100,000 ppm gNO resulted in a dose dependent cytotoxic response.
  * Higher concentrations lead to near instant cell death
  * Lower concentrations require a longer exposure period to elicit cell death
- No viable cells were detected after exposure to 50,000 ppm gaseous NO for 1 minute.
- Together with the known ability of NO to activate and recruit the immune system, these results suggest that gNO may be a potent therapeutic agent for tumor treatment across a range of tumor types.

One-way anova and Dunnet multiple comparison test, compared to non treatment, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Figure 2. Human ovarian and pancreatic cancer cell viability after exposure to gNO

Figure 3. Mouse lung, melanoma, colon and breast cancer cell viability after exposure to gNO

Figure 1. Assay scheme