The Relationship Between DNA Adduct Formation and Cytotoxicity in Human HCT116 Cells Exposed to Toxic Levels of Nitric Oxide

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Abstract

Nitric oxide (NO) is an essential biological molecule as well as a suspected mediator in the development of many cancers. Dong and Dedon studied the effects of NO in human lymphoblastoid TK6 cells, and found that steady-state concentrations of 1.75μM NO resulted in modest formation of dX (nearly undetectable), dI (~20 lesions per 10^6 nucleotides), and dU (~55 lesions per 10^6 nucleotides) after exposure to toxic doses of NO. This parallel study expanded upon that work by using more robust human colon carcinoma HCT116 cells, which allowed delivery of steady-state concentrations of 6.94μM NO. This resulted in the same undetectable formation of dX and significantly decreased formation of dI (~0.5 lesions per 10^6 nucleotides) and dU (~7.0 lesions per 10^6 nucleotides).

Background

NO is produced *in vivo* by macrophages as a cytotoxic agent in the inflammatory response. At sites of chronic inflammation, NO can reach steady-state levels of up to 10μM, and over time NO and various reactive nitrogen species (RNS) and reactive oxygen species (ROS) can accumulate in healthy tissues [1].

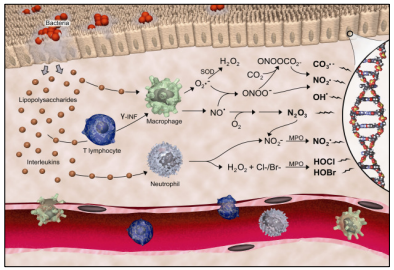

Figure 1 – Reactions of NO to Produce RNS and ROS [2]

These RNS and ROS are, in turn, known to cause the deamination products dX, dI, and dU in DNA:

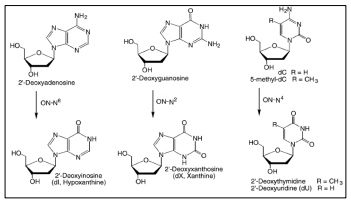

Figure 2 – Deamination Product Formation

Dong and Dedon studied NO in human lymphoblastoid TK6 cells, exposing them to steady-state concentrations of 1.75μM NO and 186μM O_2/CO_2 (near air saturation level) for up to 10h (total dose of 1260 μM·min). The result, as seen in Figure 3, was that deamination products did not significantly increase until a relatively toxic dose of NO had been delivered (diamonds represent dX, triangles represent dI, and circles represent dU) to the cells.

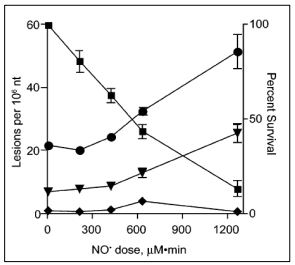

Figure 3 – Comparison of DNA Adduct Formation to TK6 Survival [3]

One possible explanation for this result is that the high sensitivity of TK6 cells to NO caused them to die before significant adduct quantities could form. To test this possibility, our study was carried out with human colon carcinoma HCT116 cells, which show a tenfold greater resistance to NO than TK6.

Cell Preparation & NO Delivery System

HAT-treated HCT116 cells were grown at 37°C in a humidified 95%/5% air/CO_2 atmosphere in McCoy's 5A Modified Medium supplemented with 10% heat-inactivated FBS; 50,000 units penicillin; 50μg streptomycin; and 5mL of 200mM L-glutamine.

NO exposures were carried out in the previously described membrane diffusion delivery system [4] shown below:

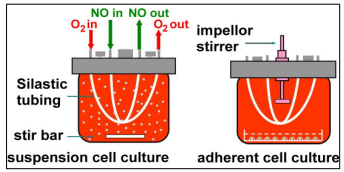

Figure 4 – Membrane Diffusion Delivery System for Nitric Oxide

NO Exposure & DNA Extraction

HCT116 cells at 5x10^6 cells/plate were exposed while stirring at 37°C to 100% NO and 50%/5%/45% O_2/CO_2/Ar through separate 7cm segments of Silastic tubing, producing calculated steady-state concentrations of 6.94μM NO and ~200μM (near air saturation level) O_2.

Based on unpublished work by Li, exposure times of 1.5h, 3h, and 6h were chosen to correspond to total NO doses below, at, and above the cytotoxic threshold (total dose of 2500 μM·min). Control cells were exposed to 100% Ar and the same O_2/CO_2/Ar mixture for 6h. All exposures were done in triplicate.

Immediately after exposure, cells were collected by centrifugation (5min @ 700 RCF), and cell pellets were washed with PBS, flash frozen, and stored at -80°C.

Cells were later resuspended in PBS and DNA was extracted with the Qiagen Genomic DNA Midi Kit.

DNA Adduct Quantification

Internal standards of 10pmol each of ^{15}N labeled dI, dX, and dU were added to each sample. To prevent adventitious DNA adduct formation, 1μL THU, 2μL coformycin, and 2μL DFX were also added.

DNA was digested with 30μL of pH 6.8 $NaC_2H_3O_2$, 10μL $ZnCl_2$, 4μL nuclease P1, and 2μL DNAse 1. After incubating for 3h at 37°C, 40μL of pH 7.8 $NaC_2H_3O_2$, 2μL alkaline phosphatase, and 2μL phosphodiesterase were added. Samples were then incubated overnight.

Concentration was measured by UV absorbance at 260nm and 280nm, and 50μg samples were taken and purified by reversed phase HPLC. Fractions containing dX, dI, and dU were collected and quantified by LC/MS/MS.

Results & Conclusion

Levels of dX, dI, and dU were plotted with HCT116 cell survival as functions of total NO dose (cell survival data based on unpublished work by Li).

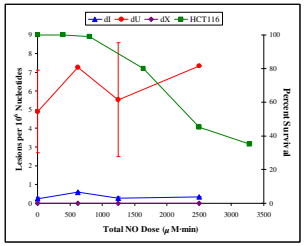

Figure 5 – Comparison of DNA Adduct Formation to HCT116 Survival

Despite being exposed to nearly twice the total dose of NO, HCT116 cells showed one hundred-fold lower levels of dI and ten-fold lower levels of dU than TK6 cells. This provides strong evidence that sensitivity to NO was not the cause of modest adduct formation in TK6.

These results also give credit to a scenario first offered by Dong and Dedon: that the rate of adduct formation *in vivo* is balanced by the rate of DNA repair. This scenario would be consistent because the more resistant HCT116 cells had more time for repair mechanisms to take effect. Above all, it is obvious that the relationship between DNA damage and cytotoxicity is highly complex.

References

[1] P.C. Dedon and S.R. Tannenbaum. *Arch. Bioch. Biophys.* 2004; 423: 12-22.
[2] Dixon, Jeff. 2004. <http://www.jeffdixon.ca/>.
[3] M. Dong and P.C. Dedon. *Chem. Res. Toxicol.* 2006; 19: 50-57.
[4] C. Wang and W.M. Deen. *Ann. Biomed. Engr.* Jan. 2003; 31(1): 65-79.