A kinetic approach for the estimation of intracellular concentrations of nitrosative species in cells challenged by nitric oxide

by

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Our understanding of how nitric oxide (NO) contributes to the initiation and progression of diseases such as cancer may benefit from determining intracellular concentrations of its reactive derivatives, for which experimental estimates are largely unavailable. During exposure of inflamed tissues to NO under physiologically relevant levels of oxygen, the potent nitrosative agent nitrous anhydride ($N_2O_3$) is formed, responsible for deamination of nucleobases in various forms of nucleic acids – yielding xanthine (X) and oxanine (O) from guanine, hypoxanthine (I) from adenine and uracil (U) from cytosine – and thus playing a multifaceted role in the pathology of chronic inflammation with implications for altered genetic content. Due to its reactivity, direct detection of the short-lived nitrosative intermediate is impossible in vitro or in vivo but, in this study, RNA nucleoside deamination products were chosen as surrogate markers for $N_2O_3$-induced damage based on the wider cellular distribution and solvent accessibility of RNA compared to genomic DNA. The objective of this thesis was to estimate the steady-state cytosolic $N_2O_3$ concentration by measuring formation rates for deamination lesions in RNA of mammalian cells stressed by NO. To establish a frame of reference in kinetic terms, purified total RNA from human lymphoblastoid TK6 cells was exposed to NO and $O_2$ at constant steady-state levels of 1.7 µM and 210 µM, respectively, in a novel NO-delivery device. Deaminated purine nucleosides were then quantified using sensitive HPLC-coupled mass spectrometry methods and analysis of the kinetics of nitrosative deamination of the RNA revealed that adenine was significantly less reactive than guanine leading to concurrent formation of inosine, oxanine and xanthosine with rate constants of $3.3 \times 10^4$, $4.8 \times 10^5$ and $1.0 \times 10^6$ M$^{-1}$s$^{-1}$, respectively. After assessing the reliability of the delivery device under conditions approximating exposure of cell cultures to NO, similar kinetic studies were performed by exposing mammalian TK6 cells to comparable levels of nitrosative stress. Smaller but measurable increases in deamination products were detected in total RNA extracted from intact cells which, when applied to expressions of the rate law in combination with the aforementioned rate constants, provided estimates for the intracellular $N_2O_3$ concentrations ranging between 0.1 and 40 fM. Parallel sets of experiments showed that a roughly 100-fold drug-induced depletion of glutathione (GSH) levels in cells prior to being challenged in the delivery device did not significantly alter the accumulation rate of rI, rX and rO, standing in contrast with previous kinetic analyses predicting GSH to be a dominant cellular scavenger of $N_2O_3$. Further calculations point to a more complicated protection against NO-induced deamination occurring in the cellular milieu with multiple scavengers likely to play an important role in mitigating the damaging effects of nitrosating species in the cytosol.