The Mechanism by which 4-Hydroxy-2,2,6,6-tetramethylpiperidene-1-oxyl (Tempol) Diverts Peroxynitrite Decomposition from Nitrating to Nitrosating Species

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Tempol is a stable nitroxide radical that has been shown to protect laboratory animals from the injury associated with conditions of oxidative and nitrosoactive stress. Tempol's protective mechanisms against reactive oxygen species have been extensively studied, but its interactions with reactive nitrogen species remain little explored. Recently, it has been shown that tempol is a potent inhibitor of peroxynitrite-mediated phenol nitration while it increases phenol nitrosation by a complex mechanism [1]. To obtain further mechanistic insights, we re-examined the interaction of $ONOO^-$ with tempol in the absence and presence of CO_2. Stopped-flow kinetic studies confirmed that tempol does not react directly with $ONOO^-$ but levels off the amount of O_2 and NO_2^- produced from $ONOO^-$ in the presence and absence of CO_2 to about 30% and 70% of initial oxidant concentration at pH 5.4, 6.4 and 7.4. Tempol inhibited phenol nitration while increasing the amounts of 4-nitrosophenol, that attained yields close to 30% of the $ONOO^-$ in the presence of CO_2 at pH 7.4. Fast-flow EPR experiments showed detectable changes in the instantaneous tempol concentration only in the presence of CO_2. Under these conditions, the instantaneous concentration of the $CO_3^{\bullet-}$ was reduced by tempol in a concentration-dependent manner. The results indicate that tempol is oxidized by peroxynitrite-derived radicals ($^{\bullet}OH$ and $CO_3^{\bullet-}$, in the absence and presence of CO_2, respectively) to the oxoammonium cation which, in turn, is reduced back to tempol while oxidizing $ONOO^-$ to O_2 and NO. The latter reacts rapidly with peroxynitrite-derived $^{\bullet}NO_2$ to produce the nitrosating species, N_2O_3. Overall, the results support a role for $ONOO^-$ and its derived radicals in the tissue pathology associated with inflammatory conditions.

[1] Carrol, R. T., *et al. Chem. Res. Toxicol.,* **13**, 294-300, 2000.