Comparing Superoxide Generation Markers by Microsomal P450 Reductase

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Superoxide anion radicals are products of several enzyme mediated reactions, such as the activities of xanthine oxidase (E.C. 1.17.3.2) and NADPH-cytochrome P450 reductase (CPR) (E.C. 1.6.2.4). Finding accurate markers for quantification of superoxide generation rates is challenging. One of the new categories of superoxide markers are water soluble tetrazoliums. In the present studies, we compared the reduction of WST-1 as a superoxide generation marker with the reduction of acetylated cytochrome c (ac-cyt c), a well-established superoxide marker, using both recombinant and purified CPR. We found that both forms of CPR reduced WST-1 in a time- and NADPH-dependent manner. Similar results were found using ac-cyt c. The reduction rate of ac-cyt c was proportional to the concentration of CPR protein; however, WST-1 reduction did not maintain a direct proportionality between the enzyme concentration and formazan formation, suggesting that CPR might catalyze WST-1 reduction in a superoxide-independent manner. This was supported by our findings that superoxide dismutase was unable to completely suppress formazan formation from WST-1 (~50% reduction) and that WST-1 increased the consumption of NADPH by CPR. Using xanthine/xanthine oxidase as a well-established superoxide generation enzyme system, both WST-1 and ac-cyt c reduction were time- and protein-dependent, and addition of SOD fully inhibited the reduction of the probes. Taken together, this data indicates that WST-1 may not be a suitable marker for quantification of superoxide formation with all enzymes, it tends to overestimate the extent of superoxide formation, at least by, NADPH-cytochrome P450 reductase. Supported by NIH P30ES005022.