Cytotoxicity and stability of new spin traps

Nadeem Khan, Carmen J. Wilmot and Harold Swartz

EPR Center for Viable Systems, Department of Diagnostic Radiology, Dartmouth Medical School, Hanover, NH 03755

The use of spin trapping recently has been extended to more complex biological systems, including functioning cells and even intact animals. This provides an opportunity to investigate the role of free radicals under the complex conditions that exist in fully functional biological systems. Such uses, however, require that the experimenter understands potential effects of the spin traps and spin adducts on the biological system and the effects of the biological system on the spin traps and spin adducts. Consequently considerable effort has been devoted to the development of new spin traps whose spin adducts may be more stable. In collaboration with the laboratories that have produced these new spin traps, we have begun a systematic evaluation of their effects on the cells and the stability of the spin adducts in the presence of functioning cells. We examined their effects on cells by measuring their effects on oxygen consumption rates of cells and the ability of cells to undergo several reproductive cycles (sufficient to form visible colonies, "clonogenicity"). We also measured the stability of their spin adducts (sulfite, hydroxyl, methyl, and hydroperoxyl radicals) in the presence of functioning cells.

At up to 25 mM concentration all of the spin traps had little or no effect on oxygen consumption rates of CHO cells; 50 mM concentrations significantly reduced the oxygen consumption rates. No significant effect on clonogenicity was observed at lower concentrations of spin traps, but 50 mM concentration significantly reduced the clone formation rate. The rate of decay off the radical adducts was determined by fitting the intensity data to appropriate decay kinetics.

While these results are incomplete, they indicate that these new spin traps are unlikely to have unacceptable cytotoxicity. It also appears that their spin adducts will be more stable in the presence of cells than for traps such as DMPO. It also should be noted, however, that these types of tests need to be carried out in the specific cell system that is going to be studied, because one cannot be sure that these effects will be the same in all cell lines.

Acknowledgement: We acknowledge Prof. Kalyanaraman, Medical College Wisconsin and Oxis International, Portland, for their kind gift of spin traps. This research was carried out at the EPR Center for Viable systems, Dartmouth Medical School, NCRR-P41 RR11602