**1**

**BLEOMYCIN ACTION ON HYDROXYL RADICAL: ITS SCAVENGING EFFECT OF ELECTRON SPIN RESONANCE STUDY.**

*Toyoko Arimoto*, Toshikazu Yoshikawa§, Masahiro Kohno#, Eiichiro Okabe¶ and Toshihiko Ozawa*. *National Institute of Radiological Sciences, Chiba , §Kyoto Prefectural University of Medicine, Kyoto 465, #Application and Research Center, JEOL Ltd., Tokyo 196, ¶Kanagawa Dental College, Kanagawa 238, Japan.*

The effects of bleomycin and the related compound peplomycin, antitumor antibiotics, on oxygen radical species generating system in vitro, were examined by electron spin resonance spin trapping method using 5, 5-dimethyl pyrroline-N-oxide (DMPO). Superoxide radical spin adduct generated from hypoxanthine plus xanthine oxidase system was not affect by bleomycin and peplomycin; however, hydroxyl radical-DMPO adduct (DMPO-OH) generated from 10 mM H2O2 plus UV irradiation system was effectively blunted by the addition of these antibiotics, in a dose-dependent manner. The IC50 values for bleomycin, peplomycin and a selective hydroxyl radical scavenger dimethylsulfoxide (DMSO) were 0.54, 0.12 and 2.4 mM, respectively, when 8.8 mM DMPO was added. To further investigate the inhibitory effects of the antibiotics used on DMPO-OH signal, we examined whether IC50 values are influenced by the concentration of DMPO added. The IC50 values for bleomycin, peplomycin and DMSO were 2.46, 0.78 and 16.1 mM, respectively, at 44 mM DMPO. These results indicate the significant scavenging effects of bleomycin and peplomycin on hydroxyl radical.

**2**

Trace Determination of Hydroxyl Radical in Biological Systems. Beibei Li, Peter L. Gutierrez*, and Neil V. Blough. Dept. of Chemistry and Biochemistry, Univ. of Maryland, College Park, MD 20742. *Greenebaum Cancer Center, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

A new, highly sensitive method for the trace determination of hydroxyl radical ($^{\bullet}OH$) production is described. This method employs the reaction between $^{\bullet}OH$ and dimethyl sulfoxide to generate quantitatively a methyl radical, which then reacts with a fluorescamine-derivatized nitroxide to produce the stable *O*-methylhydroxylamine. This *O*-methylhydroxylamine is separated by reversed phase high-performance liquid chromatography and quantified fluorometrically with a detection limit of ~250 fmoles (*Anal. Chem.* **69**:4295-4302, 1997 and *Methods Enzym.* **300**:202-216, 1999).

This method has been applied to the determination of $^{\bullet}OH$ production rates in three systems: xanthine/xanthine oxidase in the presence of iron-EDTA; NADPH-cytochrome P450 reductase in the presence of the quinone anticancer compound, diaziquone (AZQ); three mouse epidermal cell lines in the presence of AZQ. In the cell experiments, the external addition of superoxide dismutase, catalase, DTPA (a metal chelator) and Fe(III)-EDTA, as well as the measurement of probe exchange kinetics (*Methods Enzym.* **300**:202-216, 1999) has provided evidence that a portion of the $^{\bullet}OH$ is produced intracellularly. Based on our experience, this method should be broadly applicable to the determination of $^{\bullet}OH$ fluxes not only in biological relevant model systems, but also in cells.

**3**

BRAIN HYDROXYL RADICALS DURING HYPERBARIC OXYGEN CONVULSIONS

William E. Dale, Ph.D., Nana Amiridze, M.D, Ph.D., Yuhong Dang, Ph.D. and Olen R. Brown, Ph.D. Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO, USA

Microdialysis samples were collected from brain striatum of awake male rats (300-400 g) that were exposed to hyperbaric oxygen (HBO) (6 atm absolute, 5 atm gauge pressure of oxygen with $CO_2$ absorbed by soda lime). Artificial cerebrospinal fluid (CSF) containing sodium salicylate (5 mM) was perfused at 1 µl/min and collected over sequential 10 min intervals with rats breathing air, then HBO, and after decompression. Times to convulsions were recorded and CSF samples were analyzed for the reaction products of hydroxyl radicals with salicylate (i.e., 2,3- and 2,5- dihydroxybenzoic acid (DHBA)). Average time to the first convulsion was 21 min and rats convulsed an average of 4 times during 40 min in HBO. There were no significant differences in hydroxyl radical production by this protocol during any of the collection periods in air or HBO (averages in pmoles for 10 µl of all samples: 2,3-DHBA=7.0 $\pm$ 2.5 and 2,5-DHBA=11.3 $\pm$ 4.1). In future experiments we will use gas chromatography/mass spectroscopy to quantify striatal quinolinic acid (an NMDA receptor agonist and convulsant) in rats exposed to high-pressure oxygen.

**4**

**EFFECTS OF LONG-TERM SELENIUM INTAKE ON OXIDATIVE CHANGES IN BRAIN AFTER IONIZING RADIATION STRESS**

*Olga Iozanov -Stankov, Michael Simic, Ivana Djujic, Miroslav Demajo. Institute of Nuclear Sciences "Vinca".*

Nowadays, because of its special sensitivity to oxidative damage and low level in antioxidant protective agents, the special interest becomes for the role of free radicals in stress responses of the brain linking with neuronal disorders, senescence and ageing,. Selenium (Se) is also the very important part of the antioxidative defense system, and furthermore, its deficiency can predispose the living system to the oxidative stress. Blood and tissue levels of Se are related to dietary intake. In this study we exposed rats to gamma rays (single dose of 4.2 Gy, 60Co) supplemented with Se-enriched yeast (0.5 mg Se/day for 4 weeks before irradiation and after irradiation until sacrifice), or with the pure yeast - the controls. We analyzed the alterations in content of thiobarbituric acid reactive substances (TBARS) for the ratio of lipid peroxidation, as well as Se level in five areas of rat brain: front brain, hind brain, hypothalamus, pituitary and pineal gland, on 30, 150 and 240 days after irradiation. The results show that adequate intake of Se generally exhibited positive and protective effect decreasing TBARS in brain tissues in times after oxidative stress and with ageing of animals.