REACTIVE SH GROUPS IN RAT HEMOGLOBIN: ITS IMPLICATIONS IN GLUTATHIONE DEPENDENT ANTIOXIDANT AND CONJUGATIVE METABOLISM IN ERYTHROCYTE AND IN SYSTEMIC ANTIOXIDANT RESPONSES

Lusini L., 3° anno di corso del Dottorato in Medicina Molecolare - XV ciclo. Durata del Dottorato in anni: 3. Sede di servizio: Laboratorio di Farmacologia , Dipartimento di Neuroscienze, Università di Siena

The erythrocyte is constantly exposed to oxidative reactions derived from the presence of high concentrations of oxygen in the blood. Circulating endogenous and exogenous electrophiles are also a potential source of damage for a wide range of biomolecules in the erythrocyte. Glutathione is one of the most important defences against oxidative reactions in such cells, acting through a rich apparatus of enzymes. Starting from *in vitro* toxicological studies conducted in human and rat erythrocytes, our laboratory discovered and characterized at the biochemical level important features of rat haemoglobin; the high reactivity of specific cysteinyl resisues positioned in 125 chain were able to profoundly affect the antioxidant and conjugative metabolism of glutathione in rat erythrocytes.
Experiments of *in vivo* treatments presented here demonstrated that the peculiar antioxidant pathways of rat erythrocytes determined a characteristic response to the thiolic oxidant diamide. It involved the activation on an inter-organ trafficking of reducing power toward erythrocytes (which were mainly affected by the treatment) in the form of cysteine. We set up a useful system to study systemic antioxidant responses, which are to date poorly understood given the difficulties of systemic approaches. By means of vascular microsurgery techniques we made an *in vivo* time course analysis during and after the administration of diamide. Blood taken at different times was then analysed for the plasma and RBC thiolic content and redox state by HPLC techniques.
By using this experimental design we explored the mechanisms underlying antioxidant inter-organ cooperation leading to the maintenance of erythrocyte antioxidant defences. In particular the involvement of extracellular metabolism of glutathione was analysed, which depend upon the extrusion in plasma of hepatic GSH and its catabolism at tissues rich in the enzyme gamma-glutamyl transpeptidase. For this purpose acivicin, a potent and irreversible inhibitor GGT, was administered to rats prior to diamide infusion. Such pre-treatment was unable to interrupt the systemic antioxidant response, indicating that the response was independent from the extracellular metabolism of glutathione. Some experimental evidences indicate the possibility that a stimulation of the transsulfuration pathway could drive the cysteine output in the systemic antioxidant response induced by diamide.