COMPARATIVE STUDY OF THE EFFECT OF SULPHASALAZINE (SASP) AND 3,5-DIPS ON GLUTATHIONE (GSH) LEVEL IN RED CELLS OF HUMAN BLOOD *IN VITRO* AS A MODEL OF *IN VIVO* REACTION

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Abstract:

The effect of sulphasalazine (SASP), 4-pyridyl-(2) amidosulphonyl-3-carboxy-4 hyroxyazobenzol and 3,5-Diisopropylsalicylic acid (3,5- DIPS) on the chemical status and/or metabolism of the reduced status of glutathione (GSH) in red cells of human blood was examined using Ellaman's reagent method. Addition of SASP to the red cells of blood did have significant effect on GSH level in the cytosolic fraction of red cells of blood, followed by decrease in GSH level. A decrease GSH level in the cytosolic fraction of blood was further enhanced and found time dependent. Addition of 3,5-DIPS to the red cells of blood increased GSH level and found time dependent. In this paper we discussed that compounds and/or drugs which are neither thiols/GSH nor thiols/GSH-reactive may have an effect on thiols/GSH level *in vitro*, which in principle may present a model of *in vivo* reaction.

INTRODUCTION

Great interest has been focused in recent years in glutathione, (GSH) the major non-protein thiol (NPSH) in cells (Meister, 1983, Khan *et al.*, 2001) due to its varied biochemical, physiological and pharmacological activities include; drug metabolism, cellular protection against electrophilic attack of chemicals and / or its metabolites (Mitchell, 1987). GSH is known to participate critically in various detoxification reactions including in deactivation of electrophilic drugs and carcinogenic metabolites by formation of GSG-S conjugates (Khan *et al.*, 1996; Draegen *et al.*, 1976; Gurtoo *et al.*, 1981; Zitting *et al.*, 1980; Wilder *et al.*, 1982). Sulphasalazine (SASP) has been used as a second line drug which affects laboratory indices of inflammation. 3,5-Diisopropylsalicylic acid (3,5-DIPS) derivatives have various pharmacological properties (Sorenson, 1978) and it was therefore felt to compare the effects of 3,5-DIPS and SASP on GSH status in freshly isolated erythrocytes [RBC] as a model of in vivo reaction. We found that SASP affected and decreased GSH level and 3, 5-DIPS increased GSH level in RBC of blood.

Experimental

Materials

Sulphasalazine (SASP) was obtained from Pharmacia; glutathione, 5,5' dithiobis (2-nitrobenzoic acid (DTNB) Ellman's reagent and disodium hydrogen phosphate, were obtained from B.D.H. Limited. Chloroform, Ethanol from E.Merk Germany, 3, 5-Diisoprohylsalicylic acid was gifted by John Sorenson College of Pharmacy, little Rock, Arkansas, USA. Other chemicals were of reagent grade and obtained from commercial sources.

Methods

Samples of 5 ml of human venous blood of rheumatoid arthritis patients and/or volunteers treated with heparin to prevent clotting was collected. The blood was centrifuged on a Mistral 6L centrifuge at 4000rpm for 10 minutes. The plasma was removed with a Pasteur pipette. One ml of