

## EFFECT OF ISOTHIAZOLINONES ON REDOX HOMEOSTASIS OF RAT ERYTHROCYTES

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Isothiazolones are a group of chemical compounds widely used as biocidal preservatives in consumer and industrial products, by virtue of their antimicrobial properties [1]. Certain isothiazolones have recently been proposed as therapeutic agents because they inhibit telomerase, a novel and highly selective target for design of antitumor drugs [2]. They also inhibit cartilage breakdown in arthritis by blockade of a matrix metalloproteinase [3], as well as having anti-HIV activity [4].

The biological activity of these compounds is thought to originate from their interaction with intracellular thiols, such as SH groups of enzymes and receptors or simple molecules like glutathione. This interaction leads to opening of the isothiazolone ring, formation of disulfide bonds and thus impairment of cell function [5].

The aim of this study was to further investigate the interaction of isothiazolones with thiols (PSH and non protein SH groups) and modulation of redox homeostasis in rat erythrocytes. We studied the kinetics of GSH, GSSG, glutathione-protein mixed disulfides (GSSP) and PSH for 120 min after treatment with different concentrations of a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) and 2-methyl-4-isothiazolin-3-one (MI) (0.25-1 mM dose range).

After treatment with 0.25 and 0.35 mM CMI/MI, GSH and GSSP concentrations showed an immediate (5 min), dose-dependent drop and peak, respectively. This phenomenon was reversible in 120 min, concentrations returning to control values by this time. In contrast, after treatment with high doses of CMI/MI (0.5 and 1 mM) the GSH drop and GSSP peak were irreversible. Changes in GSSG concentrations were more complex: there was a peak of GSSG only when the process was reversible, though at 0.25 mM GSSG formation was immediate, whereas at 0.35 mM GSSG formation occurred after 30 min. No GSSG was formed when the process was irreversible. In these experiments, we also measured glucose consumption, availability of NADPH and changes in erythrocyte enzyme activities. Preliminary results show little or no inhibition of enzyme activities (TT and GR) whereas glucose consumption and availability of NADPH seemed to be implicated. CMI/MI may have a toxic effect on redox homeostasis, possibly due to a lack of reduced species, such as NADPH and GSH.

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