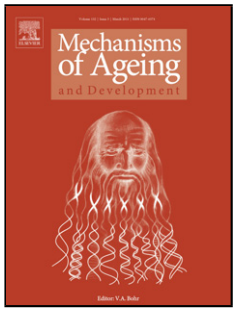

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Oxidative stress and inhibition of nitric oxide generation underlie methotrexate-induced senescence in human colon cancer cells

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Highlights

- • Methotrexate induces premature senescence in human colon cancer C85 cells
- • The drug caused dihydrofolate reductase protein stabilization
- • DNA damage occurred predominantly at the senescence initiation phase
- • ROS generation reached the highest level at the senescence maintenance phase
- • NO generation declined at the senescence maintenance phase

ABSTRACT

The response of human colon cancer C85 cells to methotrexate takes the form of reversible growth arrest of the type of stress-induced senescence. In the present study it is shown that during C85 cell progression into methotrexate-induced senescence, dihydrofolate reductase, the primary intracellular target for the drug, is stabilized at the protein level and its enzymatic activity, assayed in crude cellular extracts, decreases by 2-fold. Dihydrofolate reductase inhibition results in an increase in dihydrobiopterin level and an ultimate decrease in the tetrahydrobiopterin : dihydrobiopterin ratio in senescent cells. Endothelial nitric oxide synthase expression declines. Despite concomitant upregulation of inducible nitric oxide synthase