

Mechanism of Cell Cycle Arrest by Menadione

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Menadione (vitamin K₃, 2-methyl-1,4-naphthoquinone) has been extensively used as a model of redox-cycling quinone to study superoxide stress in mammalian systems.¹ Menadione has also attracted attention because of its significant anticancer activity *in vitro*² and *in vivo*.³ Although its effect has been attributed to the oxygen stress mechanism,⁴ it has been reported that menadione imposes lower toxicity than other clinically utilized anticancer quinones such as doxorubicin and daunorubicin.⁵

Recent study has shown differences at the level of cell cycle arrest between the response of cells to hydrogen peroxide and superoxide stress.¹¹ Thus, hydrogen peroxide and menadione treatments cause cells to arrest in G₂ and G₁, respectively. Another possible mechanism of G₁ arrest by menadione has been proposed by inactivation of cdc25A phosphatase, sulfhydryl-dependent protein, which is presumed to promote entry into S phase by acting on cdk2.⁶ Later, it has, however, been found that there was no significant selectivity associated with the inactivation of different cdc25 phosphatases (-B and -C) by menadione.⁷ However, since hydrogen peroxide and superoxide cause DNA damage mainly by the same means, through formation of the hydroxy radical, and cdc25B and -C in contrast to cdc25A, are more expressed in G₂-M,⁸ these observations cast doubt about the notion that the action of cell growth inhibition at G₁ phase simply arises from the inhibition of cdc25A phosphatase or oxidative stress.

One other interesting point is that MAP kinase activates p53 through phosphorylation, in turn, leads to the transcriptional upregulation of the cyclin-dependent kinase inhibitor, p21. The inhibitory action of p21 on cdk2 can then result in cell cycle arrest at G₁ phase.⁹ MAP kinase inactivation is mediated by dephosphorylation of the enzyme by MAP kinase phosphatase (MKP).¹⁰ Like cdc25 phosphatases, MKP is a family of dual-specificity phosphatase and can, therefore, be inactivated by menadione. If so, G₁ arrest may be resulted by p53 activation through the MAP kinase pathway.

To examine this hypothesis, MKP-1 was prepared in following manner. The entire MKP-1 was amplified from brain cDNA library by PCR by the use of the 5' oligonucleotide CACTGCTCACTTAGGACTTTCTGG and the 3' oligonucleotide TAAATAATGGAGGGGAAAGGAAAG. These fragments were inserted into *Bam*HI and *Hind*III sites of pGEX-KG, an expression vector encoding GST. The resultant plasmids were introduced into the *Escherichia coli* BL21

strain and the GST-MKP-1 was produced and purified to apparent homogeneity as described previously.⁶ Next purified phosphatase was incubated with the 40 mM *p*-nitrophenyl phosphate in 20 mM Tris (pH 8.0), 1 mM EDTA, and 0.2 mM DTT at 37 °C. Figure 1 depicts the time course of the reaction in the absence or presence of increasing concentration of menadione. In the absence of menadione, a straight line was obtained from the plot of absorbance (yield of *p*-nitrophenolate) at 410 nm *versus* time. In the presence of menadione, *p*-nitrophenylate formation decreased with increasing concentration of menadione. This result indicated the menadione inactivates the GST-MKP-1. Like cdc25 phosphatase inactivation by menadione, the activity of inactivated enzyme with menadione did not return after dialysis, suggesting an irreversible inactivation process.⁶ Moreover protection from inhibition in the presence of the competitive reversible inhibitor, arsenate, showed that menadione reacts with the active site of the enzyme (*data not shown*). This experiment is consistent with the observations of Carr and co-workers, who found that vitamin K-related quinoid compound stimulates MAP kinase phosphorylation, which may be caused by MKP inactivation.¹¹

Previously, in the experiment reported by others superoxide dismutase did not antagonize the growth inhibitory effects by menadione.¹² This result can be understood as a consequence of the one-electron reduction potential of menadione. Quinone redox cycling implies autoxidation of quinone reduction products. During autoxidation, two one-electron transfer steps with formation of semiquinone inter-

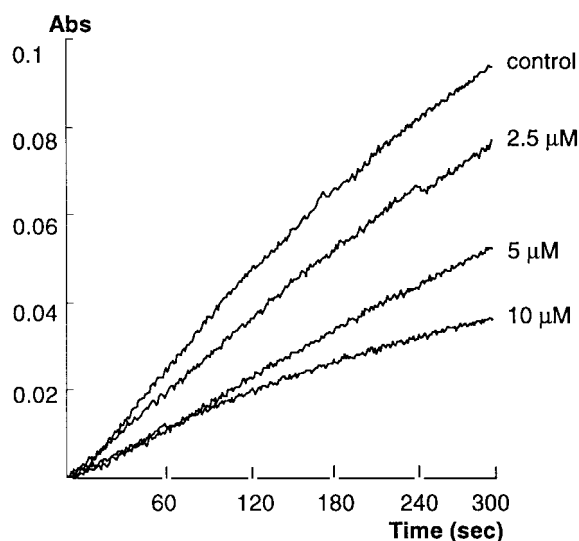
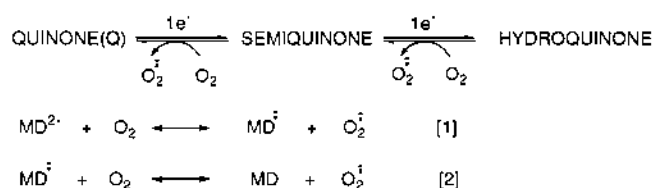


Figure 1. Time course of the reaction of MKP-1 in the absence or presence of menadione.

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Scheme 1. Bioreductive Activation of Menadione (MD) by two electrons.

mediates. (reactions 1 and 2). Values of the one-electron reduction potential of menadione and the reduction potential for addition of the second electron to menadione are -203 mV and +193 mV, respectively.¹³ Comparing these values with that of oxygen (-330 mV), equilibriums of reaction 1 and 2 are further displaced to the left, thus disfavoring superoxide formation.¹³ Moreover, the generated toxic oxygen species induced by menadione should undergo a Fenton reaction (iron-dependent) to induce DNA damage in cells. However, the fact that the toxic oxygen species, inducing most of the DNA breakages in menadione-treated Chinese hamster ovary cells, are not responsible for menadione's cytotoxicity was indicated by the co-incubation of iron-chelator.¹⁴

In conclusion, we report here that redox cycling does not play a critical role in menadione-induced cell cycle arrest in cells. Since menadione showed no inactivation of protein tyrosine phosphatases and protein serine/threonine phosphatase,⁷ inactivation of dual-specificity phosphatases is likely to be menadione's primary mechanism of action in cell growth inhibition. Therefore, the role of menadione as the superoxide generator in cells should be re-evaluated.

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