Letter to the Editor

The Role of ROS in Hydroquinone-induced Inhibition of K562 Cell Erythroid Differentiation*

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The role of ROS in hydroquinone-induced inhibition of K562 cell erythroid differentiation was investigated. After K562 cells were treated with hydroquinone for 24 h, and hemin was later added to induce erythroid differentiation for 48 h, hydroquinone inhibited hemin-induced hemoglobin synthesis and mRNA expression of γ-globin in K562 cells in a concentration-dependent manner. The 24-h exposure to hydroquinone also caused a concentration-dependent increase at an intracellular ROS level, while the presence of N-acetyl-L-cysteine prevented hydroquinone- induced ROS production in K562 cells. The presence of N-acetyl-L-cysteine also prevented hydroquinone inhibiting hemin-induced hemoglobin synthesis and mRNA expression of γ-globin in K562 cells. These evidences indicated that ROS production played a role in hydroquinone-induced inhibition of erythroid differentiation.

Exposure to benzene causes extensive toxic effects, especially hematotoxicity. It is widely accepted that benzene requires metabolism to induce its toxic effects, and hydroquinone is considered as one of its major toxic metabolites. In addition, hydroquinone as a widely used chemical often leads to occupational and environmental exposure. Hydroquinone has been demonstrated to display myelotoxic, hematotoxic, hepatotoxic, and nephrotoxic properties. Hydroquinone is easily oxidized to form reactive semiquinone and quinone, which leads to reactive oxygen species (ROS) production[1]. It was shown that hydroquinone-induced apoptosis was accompanied by elevated ROS levels, but the hydroquinone-induced apoptosis and activation of caspases were reversed by pre-treatment with antioxidants, such as N-acetyl-L-cysteine (NAC)[2-3]. Our recent study showed that benzene metabolites demonstrated different effects on erythroid differentiation of K562 cells, and hydroquinone inhibited their erythroid differentiation at a non-cytotoxic concentration[4-6]. Badham and Winn (2010) also found that exposure to hydroquinone reduced the percentage of differentiated HD3 cells, which was associated with an increase in ROS[7]. The present study was aimed to elucidate the role of ROS in hydroquinone-induced inhibition of K562 cells erythroid differentiation.

At first, we determined hemin-induced hemoglobin synthesis in hydroquinone-pretreated K562 cells. If K562 cells were induced by hemin, the percentage of hemoglobin-positive cells was 41.0%, but the percentage of hemoglobin-positive cells was less than 5% in no-induced K562 cells. When K562 cells were pre-treated with hydroquinone of 5 μmol/L, 10 μmol/L, or 20 μmol/L for 24 h, and then stimulated by hemin, as shown in Figure 1a, the percentages of hemoglobin-positive cells decreased to 39.6%, 35.8%, and 32.7%, respectively, showing a concentration-dependent decrease. The γ-globin gene encoded a subunit of hemoglobin, and hemin was demonstrated to induce γ-globin expression in K562 cells. We next determined the mRNA level of γ-globin gene in K562 cells that were treated with hydroquinone. Compared to no-induced K562 cells, hemin-induced K562 cells showed a 4.1-fold increase in γ-globin mRNA expression. When K562 cells were pre-treated with 5 μmol/L, 10 μmol/L, or 20 μmol/L hydroquinone for 24 h, the hemin-induced mRNA expression of γ-globin genes demonstrated only 3.1-, 2.5-, and 1.6-fold increases, respectively, showing a concentration-dependent decrease (Figure 1b). Our previous results showed that at the exposure to 40 μmol/L hydroquinone, the hemin-induced hemoglobin synthesis showed a time-dependent decrease, and hemin-induced mRNA expression of α-, β-, and γ-globins was also inhibited[4]. Present results confirmed that hydroquinone inhibited the erythroid differentiation potency of K562 cells.

Hydroquinone is easily oxidized to form ROS, which leads to oxidized stress[1]. DCFH-DA was used to monitor the intracellular ROS level in K562 cells

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after exposure to hydroquinone. As expected, compared to the control K562 cells, the K562 cells exposed to 5, 10, 20 μmol/L hydroquinone for 24 h showed a significant increase at intracellular ROS levels in a concentration-dependent manner (Figure 2a). NAC is a commonly used antioxidant. It interacts directly with ROS to functions as a scavenger of oxygen free radicals. The antioxidant action of NAC is also attributed to its ability to serve as a precursor to GSH. We investigated the effect of antioxidant NAC at intracellular ROS levels in hydroquinone-exposed K562 cells. Consistently, as shown in Figure 2b, the addition of NAC significantly prevented hydroquinone increasing intracellular ROS levels.

It has been reported that hydroquinone-induced genotoxicity and apoptosis were associated with ROS production, and NAC has been found to abolish hydroquinone-induced apoptosis[3]. Antioxidant glutathione could inhibit hydroquinone-induced ROS production and DNA fragmentation effectively[8]. Badham and Winn (2010) found that exposure to hydroquinone inhibited differentiation of HD3 cells, which could be effectively blocked by exogenous SOD[7]. We used NAC as antioxidant to investigate the relationship between ROS production and inhibition of erythroid differentiation. As shown in Figure 3a, exposure to 20 μmol/L hydroquinone for 24 h significantly inhibited hemin-induced hemoglobin

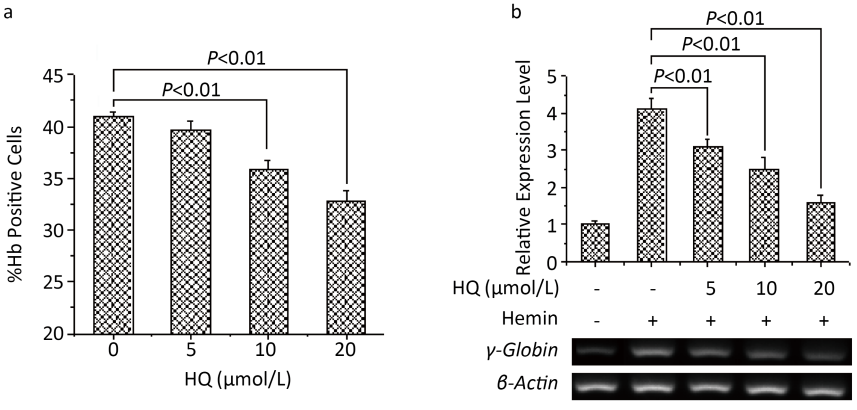

Figure 1. Hydroquinone inhibited hemin-induced erythroid differentiation in K562 cells. After K562 cells were treated with 5-20 μmol/L hydroquinone (HQ) for 24 h, 40 μmol/L hemin was added to induce erythroid differentiation for 48 h. (a) Hemoglobin synthesis was estimated by benzidine staining. Each datum represents the mean±SD of six independent experiments. (b) The mRNA level of γ-globin gene was analyzed with RT-PCR. The semiquantitative data were from three independent experiments (mean±SD).

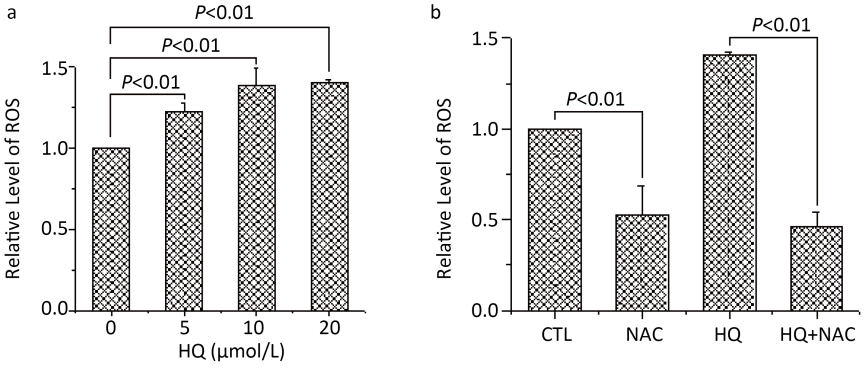

Figure 2. The effects of NAC on hydroquinone-induced increase in intracellular ROS in K562 cells. (a) Hydroquinone (HQ) induced an increase in intracellular ROS in K562 cells. K562 cells were exposed to hydroquinone (0, 5, 10, 20 μmol/L) for 24 h. Intracellular ROS was indicated using DCFDA and analyzed by flow cytometry. (b) Hydroquinone-induced increase in intracellular ROS was prevented by NAC. K562 cells were treated with 20 μmol/L hydroquinone and 10 mmol/L NAC for 24 h, and the intracellular ROS was analyzed. CTL means control. Each datum represents the mean±SD of six independent experiments.

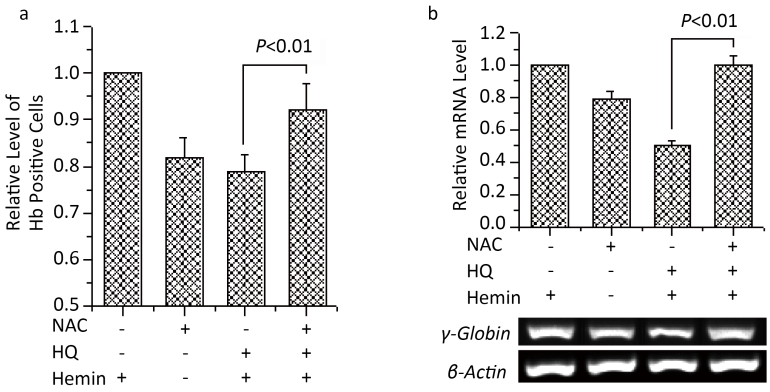

Figure 3. NAC eliminated hydroquinone-induced inhibition of erythroid differentiation. K562 cells were treated with 20 μmol/L hydroquinone and 10 mmol/L NAC for 24 h, and 40 μmol/L hemin was added to induce erythroid differentiation for 48 h. (a) Hemoglobin synthesis in K562 cells was estimated by benzidine staining. Each datum represents the mean±SD of six independent experiments. (b) The mRNA level of γ-globin gene was analyzed with RT-PCR. The data were semiquantitative from three independent experiments (mean±SD).

synthesis of K562 cells by 21%, and the addition of 10 mmol/L NAC significantly prevented the decrease in hemin-induced hemoglobin synthesis of K562 cells. Furthermore, when K562 cells were pretreated with 20 μmol/L hydroquinone for 24 h, the hemin-induced mRNA expression of γ-globin gene decreased by 50%, whereas the presence of NAC blocked the inhibitory effect of hydroquinone (Figure 3b). These results suggested that ROS production was involved in hydroquinone-induced inhibition of erythroid differentiation.

The exposure to benzene and its metabolites has been shown to increase the expression, activity and phosphorylation of c-Myb in K562 cells and HD3 cells via the ROS-activated NF-κB/Pim-1 pathway[1]. The transcription factor c-Myb is required for expansion of erythroid progenitors, but down-regulation is needed to permit differentiation into mature erythrocytes[9]. Consistently, c-Myb in cultured pro-erythroblasts and K562 cells is rapidly down-regulated during erythroid differentiation[10]. This finding suggests that hydroquinone-induced ROS production is likely to activate c-Myb, which blocks the process of erythroid differentiation of K562 cells.

In summary, hydroquinone has inhibited hemin-induced erythroid differentiation of K562 cells, which is associated with ROS production. However, further studies are warranted to clarify the exact mechanisms, such as the roles of c-Myb and other redox-sensitive signaling molecules.

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