

CHAPTER III

RESULTS

3.1 Effects of G-CSF on mitochondrial swelling

Transmission electron microscope was used for the identification of the cardiac mitochondria (Figure 1). The freshly isolated cardiac mitochondria is shown in **Figure 3-1 A**. Application of 2 mM H₂O₂ to cardiac mitochondria caused mitochondrial swelling with markedly unfold cristae (**Figure 3-1 B**). When G-CSF at 50 ng/ml was applied to cardiac mitochondria 30 min prior to H₂O₂ application, the morphology of cardiac mitochondria was well preserved, indicating the effectiveness of G-CSF in preventing morphological change in cardiac mitochondria caused by H₂O₂ application (**Figure 3-1 C**). Mitochondrial swelling was quantitatively assessed by the reduction in the absorbance of the mitochondrial suspension. Application of H₂O₂ to mitochondria resulted in a decrease in the absorbance, suggesting that H₂O₂ could significantly induce mitochondrial swelling. In this study, we investigated optimal time for effective G-CSF effects (**Figure 3-2**). From this figure showed that G-CSF pretreated mitochondria for 30 and 60 min before H₂O₂ application can effectively prevent mitochondrial swelling. In this study, G-CSF pretreated mitochondria for 30 min before H₂O₂ application was used in investigating effects of G-CSF. From our result suggested that G-CSF at 25 ng/ml could not prevent a decrease in the absorbance, whereas G-CSF at 50, 100 and 200 ng/ml could effectively prevent a reduction in the absorbance after H₂O₂ application (**Figure 3-3**). From this kinetic study, a time point at 4 min after H₂O₂ application was chosen to

investigate the protective effect of G-CSF. Without H₂O₂, G-CSF itself did not alter the absorbance, compared to the control group (**Figure 3-4**). When G-CSF was applied to mitochondria prior to H₂O₂ application, G-CSF at concentration of 50, 100 and 200 ng/ml could significantly reduce mitochondrial swelling, compared to that in H₂O₂ group. The absorbance measured in these mitochondria pretreated with G-CSF was not different from that in the control (G0) group (**Figure 3-4**).

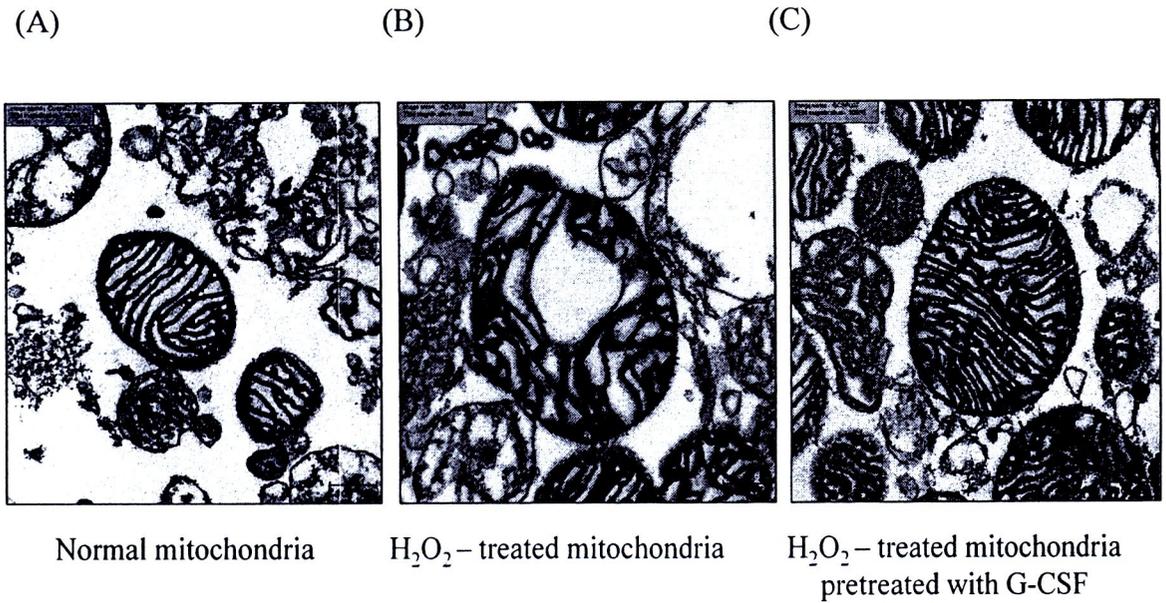


Figure 3-1

The identification of the cardiac mitochondria with electron microscopy, (A) normal mitochondria, (B) cardiac mitochondria treated with H_2O_2 , (C) cardiac mitochondria pretreated with G-CSF at 50 ng/ml followed by H_2O_2 application.

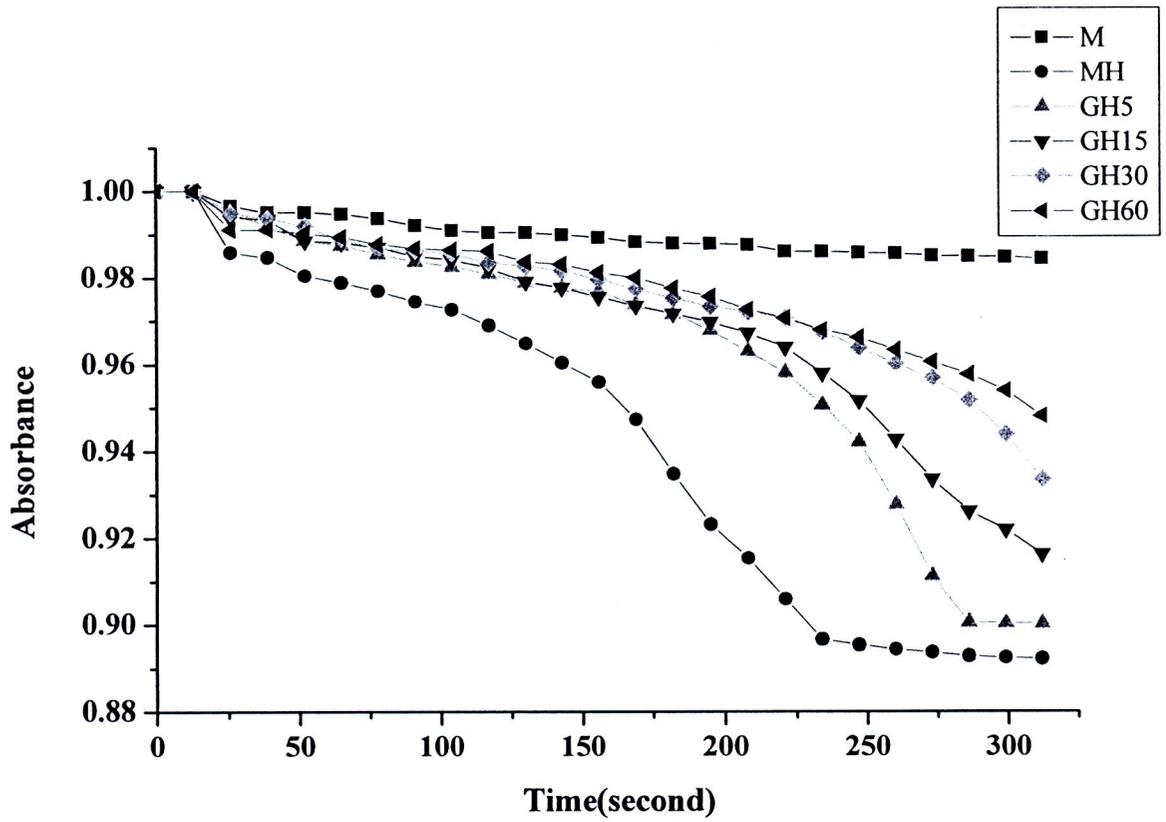


Figure 3-2

The vary time of G-CSF effect on mitochondrial swelling, G-CSF at 100 ng/ml were added before application of H_2O_2 . Application time of G-CSF was varied indicated by 5, 15, 30 and 60 min before H_2O_2 was added, respectively.

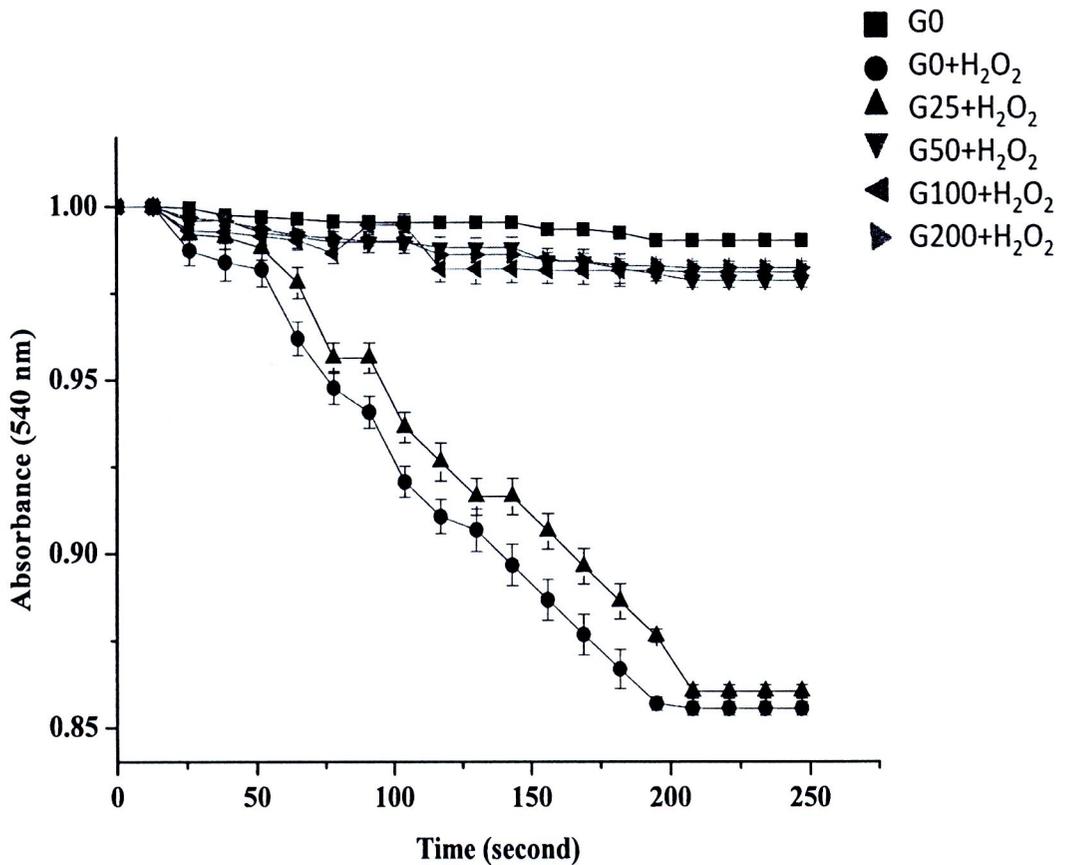


Figure 3-3

The kinetic study of the effects of G-CSF on mitochondrial swelling. Mitochondrial swelling was quantitatively assessed by the reduction in the absorbance of the mitochondrial suspension. G0 = cardiac mitochondria not treated by H₂O₂ or G-CSF, (G0 + H₂O₂) = cardiac mitochondria treated with H₂O₂, (G25, 50, 100 and 200 + H₂O₂) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H₂O₂ application, respectively.

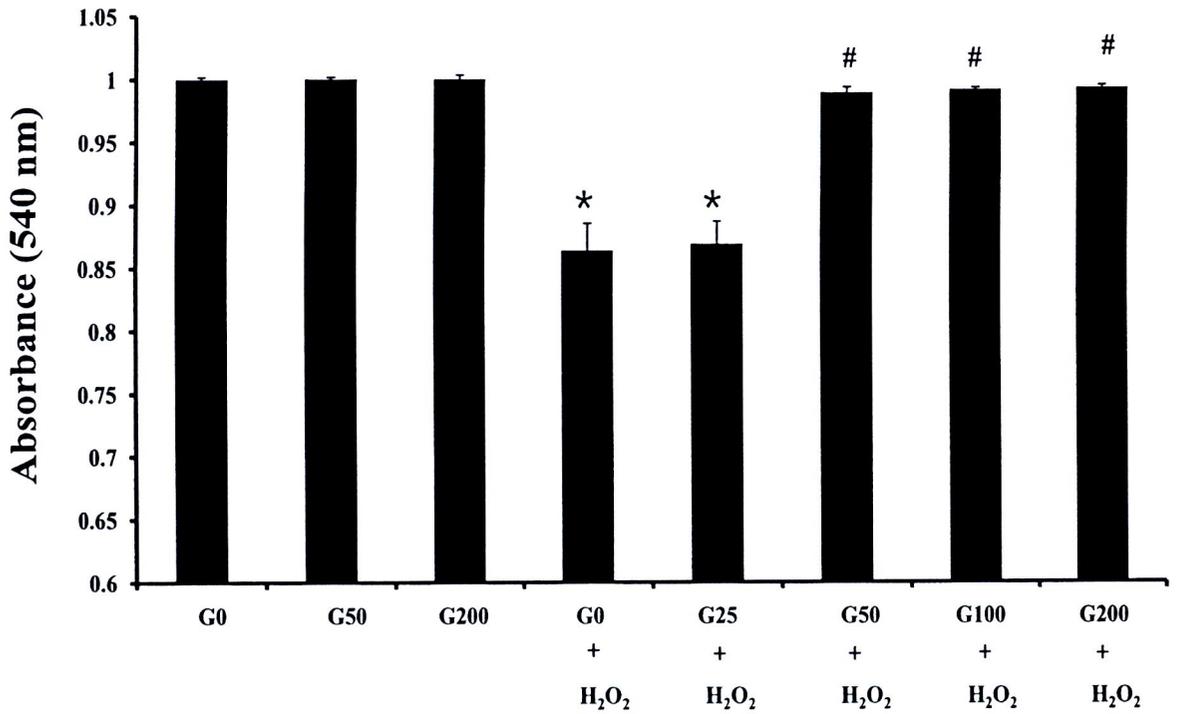


Figure 3-4

The effects of G-CSF on mitochondrial swelling after 4-min H₂O₂ application. G0 = cardiac mitochondria not treated by H₂O₂ or G-CSF, G50 and G200 = cardiac mitochondria treated with G-CSF 50 and 200 ng/ml, respectively, (G0 + H₂O₂) = cardiac mitochondria treated with H₂O₂, (G25, 50, 100 and 200 + H₂O₂) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H₂O₂ application, respectively. **P* < 0.05 vs. G0 group, #*P* < 0.05 vs. (G0 + H₂O₂) group.



3.2 Effects of G-CSF on ROS production

At baseline, ROS level in cardiac mitochondria treated with G-CSF (50 and 200 ng/ml) groups were not different from the control group (**Figure 3-5**). When H_2O_2 was applied to the mitochondrial suspension, the level of ROS was significantly induced, compared to the control group. However, when G-CSF was applied to mitochondria prior to H_2O_2 application, G-CSF (25, 50, 100 and 200 ng/ml) significantly reduced ROS level, compared to the H_2O_2 group.

3.3 Effects of G-CSF on mitochondrial membrane potential changes ($\Delta\Psi_m$)

At baseline, the $\Delta\Psi_m$ in G-CSF (50 and 200 ng/ml) group was not significantly increased, compared to the control group (**Figure 3-6**). When H_2O_2 was added on mitochondrial suspension, H_2O_2 can significantly increase mitochondrial depolarization to compare control group. However, when G-CSF was applied to mitochondria prior to H_2O_2 application, G-CSF (50, 100 and 200 ng/ml) significantly reduced mitochondrial membrane potential changes to compare H_2O_2 group.

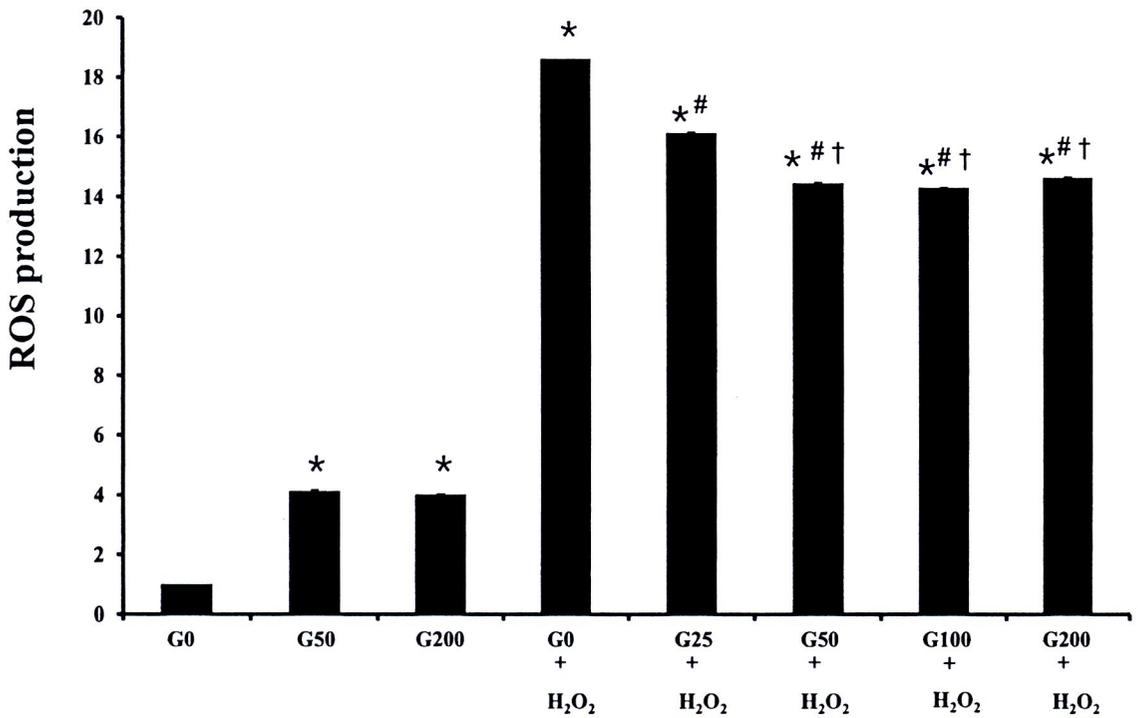


Figure 3-5

The effects of G-CSF on ROS production. G0 = cardiac mitochondria not treated by H₂O₂ or G-CSF, G50 and G200 = cardiac mitochondria treated with G-CSF 50 and 200 ng/ml, respectively, (G0 + H₂O₂) = cardiac mitochondria treated with H₂O₂, (G25, 50, 100 and 200 + H₂O₂) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H₂O₂ application, respectively **P* < 0.05 vs. G0 group, #*P* < 0.05 vs. (G0 + H₂O₂) group.

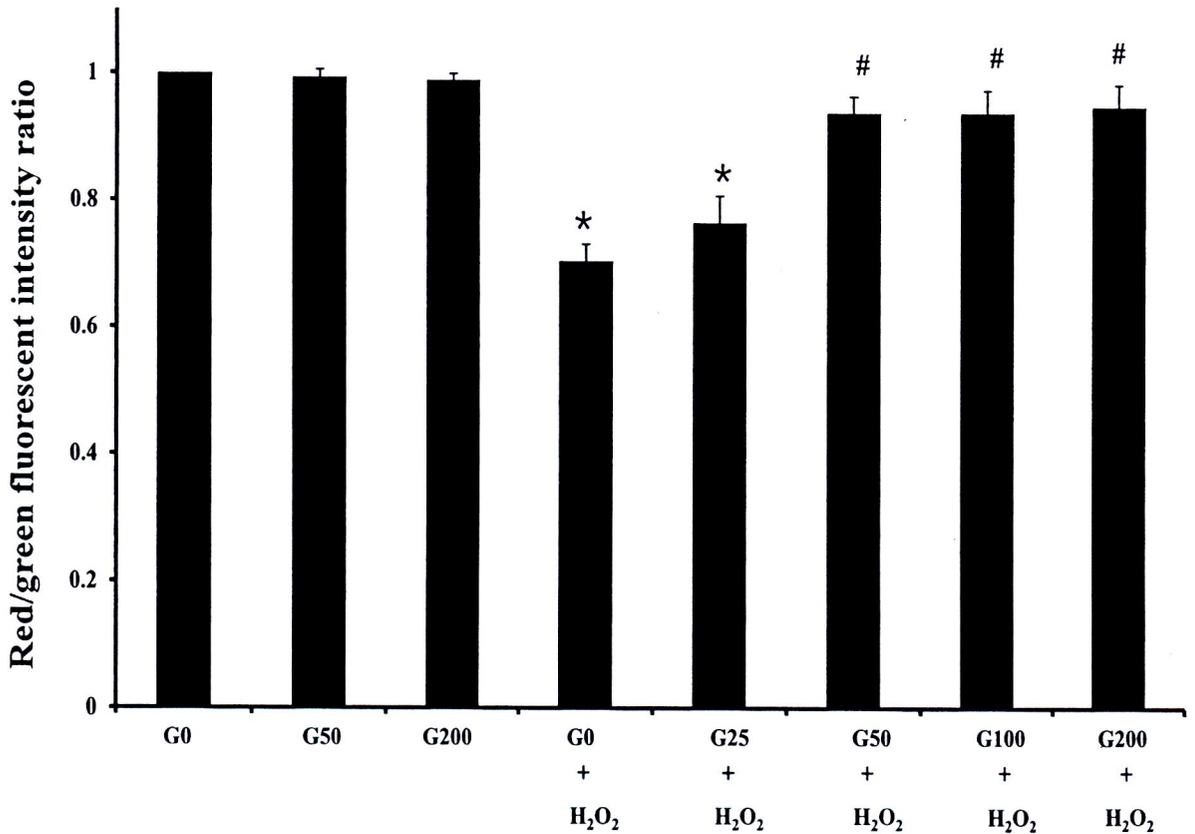


Figure 3-6

The effects of G-CSF on mitochondrial membrane potential ($\Delta\Psi_m$) changes. G0 = cardiac mitochondria not treated by H₂O₂ or G-CSF, G50 and G200 = cardiac mitochondria treated with G-CSF 50 and 200 ng/ml, respectively, (G0 + H₂O₂) = cardiac mitochondria treated with H₂O₂, (G25, 50, 100 and 200 + H₂O₂) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H₂O₂ application, respectively **P* < 0.05 vs. G0 group, #*P* < 0.05 vs. (G0 + H₂O₂) group.

3.4 Effect of G-CSF, CsA and CDP on cardiac mitochondrial swelling

At baseline, G-CSF at 50 ng/ml (i.e. G50), CsA and CDP did not cause any changes in the absorbance of cardiac mitochondria (**Figure 3-7**). G-CSF at 50 ng/ml was chosen since it could effectively prevent the absorbance reduction after H₂O₂ application as demonstrated in the previous protocol. When H₂O₂ was applied to mitochondrial suspension, the absorbance was markedly decreased compared to the control group. However, mitochondria pretreated with G-CSF or CsA or CDP prior to H₂O₂ application could markedly attenuate the reduction in the absorbance, compared to the H₂O₂ group. No difference was found among these G-CSF, CsA and CDP pretreated groups. Furthermore, a combination of (G-CSF+CsA) or (G-CSF+CDP) or (CsA+CDP) or (G-CSF+CsA+CDP) could effectively prevent the absorbance reduction in cardiac mitochondria caused by H₂O₂. The measured absorbance was not different among these groups, and was not different from that in the control group (**Figure 3-7**).

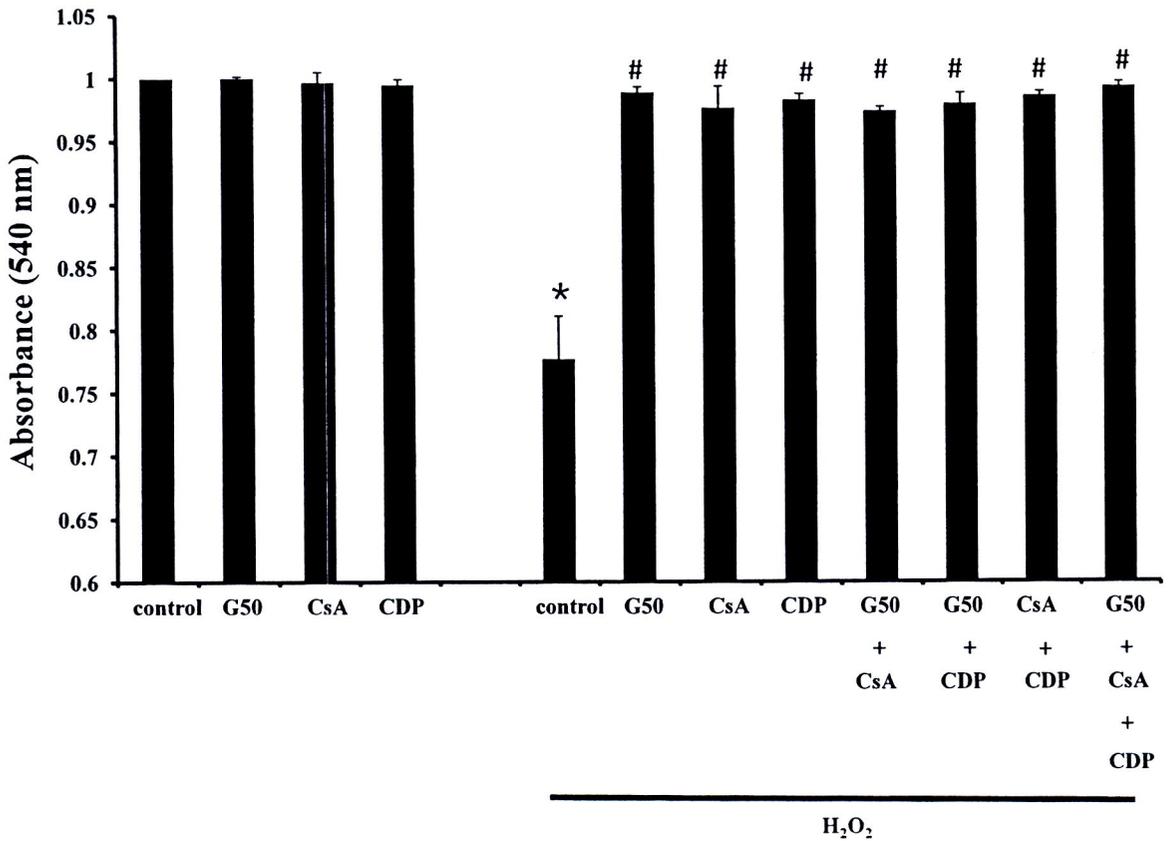


Figure 3-7

The effects of G-CSF, CsA and CDP on mitochondrial swelling at 4 min. Control = cardiac mitochondria not treated by H₂O₂ or G-CSF. G50, CsA and CDP = cardiac mitochondria treated with G-CSF 50 ng/ml, CsA and CDP, respectively, (control + H₂O₂) = cardiac mitochondria treated with H₂O₂, (G50, CsA or CDP + H₂O₂) = mitochondria pretreated with G-CSF 50 ng/ml, CsA or CDP followed by H₂O₂ application, respectively. **P* < 0.05 vs. control group, #*P* < 0.05 vs. H₂O₂ group.

3.5 Effects of G-CSF, CsA and CDP on ROS production

At baseline, G-CSF at 50 ng/ml and CsA caused a slight increase in ROS production in isolated cardiac mitochondria (**Figure 3-8**). The ROS level was markedly increased in mitochondria treated with H₂O₂. However, in cardiac mitochondria pretreated with G-CSF or CsA or CDP prior to H₂O₂ application, the ROS level was significantly decreased, compared to the H₂O₂ group. However, the reduction of ROS level among these groups was not equal. CsA could prevent ROS production with greatest efficacy, followed by CDP and G-CSF, respectively. In addition, when H₂O₂ was added to mitochondria pretreated with (G-CSF+CsA) or (G-CSF+CDP) or (CsA+CDP) or (G-CSF+CsA+CDP) groups, the ROS level was significantly decreased, compared to that in the H₂O₂ group. However, the level of ROS production was not different among these combined treatment groups. Furthermore, the reduction in ROS level in these combined treatment groups was similar to that in CsA+H₂O₂ group (**Figure 3-8**).

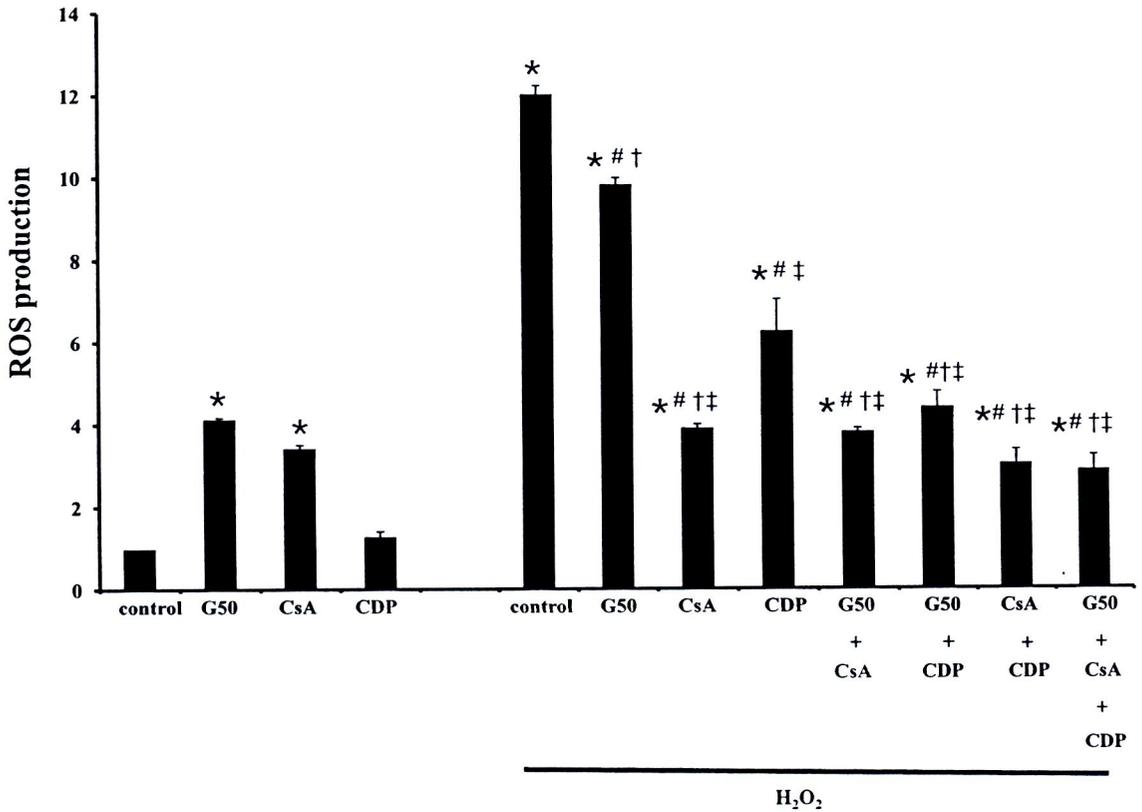


Figure 3-8

The effects of G-CSF, CsA and CDP on ROS production. Control = cardiac mitochondria not treated by H₂O₂ or G-CSF. G50, CsA and CDP = cardiac mitochondria treated with G-CSF 50 ng/ml, CsA and CDP, respectively, (control + H₂O₂) = cardiac mitochondria treated with H₂O₂, (G50, CsA or CDP + H₂O₂) = mitochondria pretreated with G-CSF 50 ng/ml, CsA or CDP followed by H₂O₂ application, respectively. **P* < 0.05 vs. control group, #*P* < 0.05 vs. H₂O₂ group, †*P* < 0.05 vs. (CDP + H₂O₂), ‡*P* < 0.05 vs. (G-CSF + H₂O₂).

3.6 Effects of G-CSF, CsA and CDP on mitochondrial membrane potential

($\Delta\Psi_m$) changes

At baseline, G-CSF at 50 ng/ml and CDP alone did not cause any changes in $\Delta\Psi_m$ in cardiac mitochondria. However, CsA alone caused a slight decrease in $\Delta\Psi_m$ (mitochondrial depolarization) (**Figure 3-9**). When H_2O_2 was added to cardiac mitochondria, $\Delta\Psi_m$ was markedly altered, compared to the control group. However, application of H_2O_2 to mitochondria pretreated with G-CSF or CsA or CDP could not alter the $\Delta\Psi_m$. When H_2O_2 was added to mitochondria pretreated with (G-CSF+CsA) or (G-CSF+CDP) or (CsA+CDP) or (G-CSF+CsA+CDP), the $\Delta\Psi_m$ was also not altered, compared to that in the H_2O_2 group. No difference was found for $\Delta\Psi_m$ among these combined treatment groups. Furthermore, the $\Delta\Psi_m$ in these groups was not different from that in the control group (**Figure 3-9**).

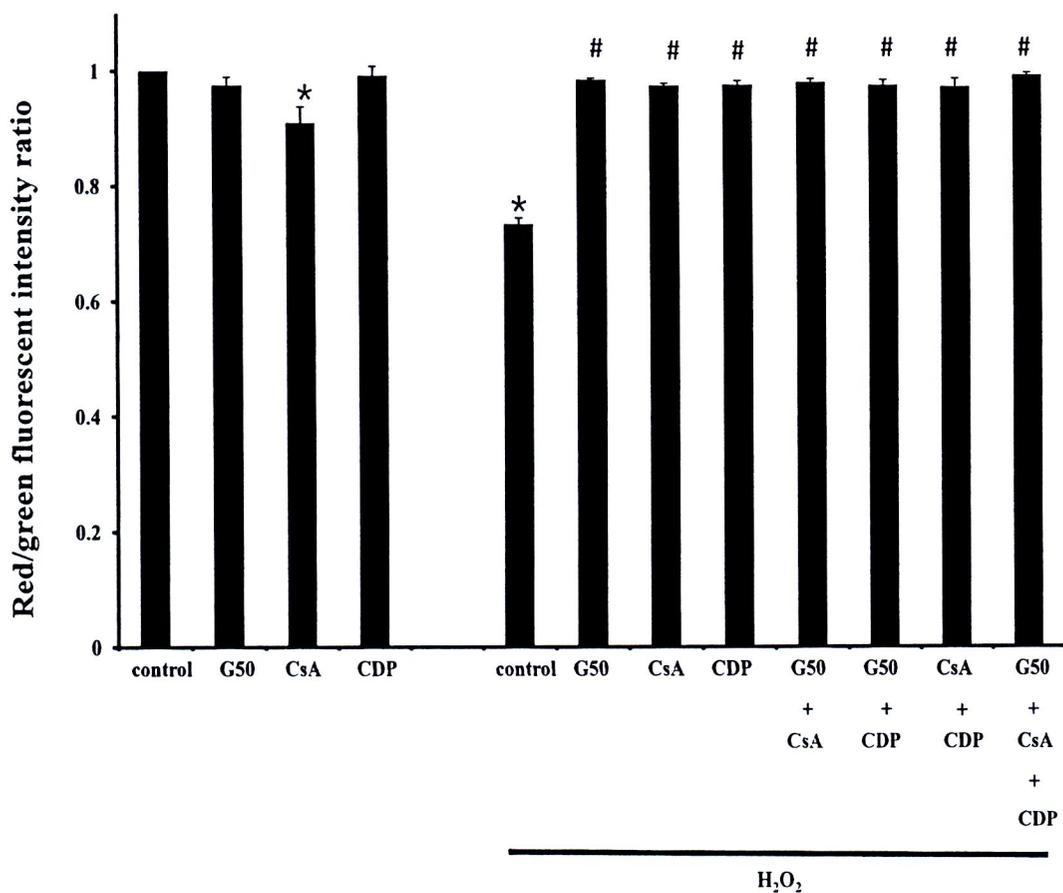


Figure 3-9

The effects of G-CSF, CsA and CDP on mitochondrial membrane potential ($\Delta\Psi_m$) changes. Control = cardiac mitochondria not treated by H_2O_2 or G-CSF. G50, CsA and CDP = cardiac mitochondria treated with G-CSF 50 ng/ml, CsA and CDP, respectively, (control + H_2O_2) = cardiac mitochondria treated with H_2O_2 , (G50, CsA or CDP + H_2O_2) = mitochondria pretreated with G-CSF 50 ng/ml, CsA or CDP followed by H_2O_2 application, respectively. * $P < 0.05$ vs. control group, # $P < 0.05$ vs. H_2O_2 group.