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 H_2O_2 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 H_2O_2 application, the morphology of cardiac mitochondria was well preserved, indicating the effectiveness of G-CSF in preventing morphological change in cardiac mitochondria caused by H_2O_2 application **(Figure 3-1 C)**. Mitochondrial swelling was quantitatively assessed by the reduction in the absorbance of the mitochondrial suspension. Application of H_2O_2 to mitochondria resulted in a decrease in the absorbance, suggesting that H_2O_2 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 H_2O_2 application can effectively prevent mitochondrial swelling. In this study, G-CSF pretreated mitochondria for 30 min before H_2O_2 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 H_2O_2 application **(Figure 3-3)**. From this kinetic study, a time point at 4 min after H_2O_2 application was chosen to

investigate the protective effect of G-CSF. Without H_2O_2, 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 H_2O_2 application, G-CSF at concentration of 50, 100 and 200 ng/ml could significantly reduce mitochondrial swelling, compared to that in H_2O_2 group. The absorbance measured in these mitochondria pretreated with G-CSF was not different from that in the control (G0) group **(Figure 3-4)**.

(A) (B) (C)

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.

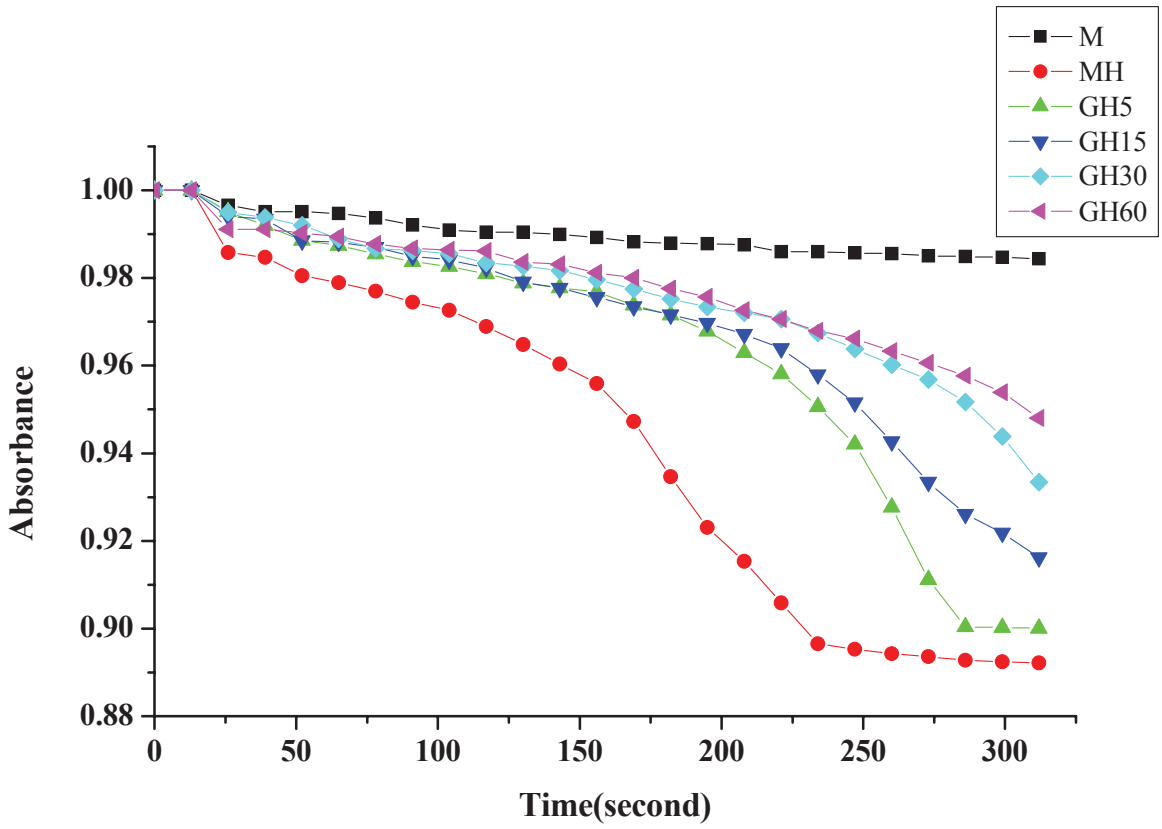

Figure 3-2

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 H_2O_2 or G-CSF, (G0 + H_2O_2) = cardiac mitochondria treated with H_2O_2, (G25, 50, 100 and 200 + H_2O_2) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H_2O_2 application, respectively.

Figure 3-4

The effects of G-CSF on mitochondrial swelling after 4-min H_2O_2 application. G0 = cardiac mitochondria not treated by H_2O_2 or G-CSF, G50 and G200 = cardiac mitochondria treated with G-CSF 50 and 200 ng/ml, respectively, (G0 + H_2O_2) = cardiac mitochondria treated with H_2O_2, (G25, 50, 100 and 200 + H_2O_2) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H_2O_2 application, respectively. $^{*}P < 0.05$ vs. G0 group, $^{\#}P < 0.05$ vs. (G0 + H_2O_2) 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 (ΔΨm)

At baseline, the ΔΨm 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 H_2O_2 or G-CSF, G50 and G200 = cardiac mitochondria treated with G-CSF 50 and 200 ng/ml, respectively, (G0 + H_2O_2) = cardiac mitochondria treated with H_2O_2, (G25, 50, 100 and 200 + H_2O_2) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H_2O_2 application, respectively *$P < 0.05$ vs. G0 group, $^{\#}P < 0.05$ vs. (G0 + H_2O_2) group.

Figure 3-6

The effects of G-CSF on mitochondrial membrane potential ($\Delta\Psi m$) changes. G0 = cardiac mitochondria not treated by H_2O_2 or G-CSF, G50 and G200 = cardiac mitochondria treated with G-CSF 50 and 200 ng/ml, respectively, (G0 + H_2O_2) = cardiac mitochondria treated with H_2O_2, (G25, 50, 100 and 200 + H_2O_2) = mitochondria pretreated with G-CSF at 25, 50, 100 and 200 ng/ml followed by H_2O_2 application, respectively $^{*}P < 0.05$ vs. G0 group, $^{\#}P < 0.05$ vs. (G0 + H_2O_2) 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 H_2O_2 application as demonstrated in the previous protocol. When H_2O_2 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 H_2O_2 application could markedly attenuate the reduction in the absorbance, compared to the H_2O_2 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 H_2O_2. 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 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.

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 H_2O_2. However, in cardiac mitochondria pretreated with G-CSF or CsA or CDP prior to H_2O_2 application, the ROS level was significantly decreased, compared to the H_2O_2 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 H_2O_2 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 H_2O_2 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+H_2O_2 group **(Figure 3-8)**.

Figure 3-8

The effects of G-CSF, CsA and CDP on ROS production. 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, †$P < 0.05$ vs. (CDP + H_2O_2), $^{\ddagger}P < 0.05$ vs. (G-CSF + H_2O_2).

3.6 Effects of G-CSF, CsA and CDP on mitochondrial membrane potential (ΔΨm) changes

At baseline, G-CSF at 50 ng/ml and CDP alone did not cause any changes in ΔΨm in cardiac mitochondria. However, CsA alone caused a slight decrease in ΔΨm (mitochondrial depolarization) **(Figure 3-9)**. When H_2O_2 was added to cardiac mitochondria, ΔΨm 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 ΔΨm. 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 ΔΨm was also not altered, compared to that in the H_2O_2 group. No difference was found for ΔΨm among these combined treatment groups. Furthermore, the ΔΨm 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.