出國報告 (出國類別: 進修)

粒腺體在前置訓練的角色-缺血後制約訓練可減少短暫大腦缺血傷害及粒線體功能受損

(The Role of mitochondria in preconditioning- ISCHEMIC POSTCONDITONING PROTECTS THE BRAIN FROM TRANSIENT GLOBAL CEREBRAL ISCHEMIA AND MITIGATES MITOCHONDRIA DYSFUNCTION)

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出國期間:96 年 8 月 14 日至 97 年 8 月 13 日

報告日期: 97 年 9 月 22 日

摘要:

粒線體的功能調控是引起缺血灌流傷害的重要病態生理機制之一。 故，研究以粒線體爲治療標的，期減少大腦等器官的缺血灌流傷害。缺血後制約訓練係改變發生缺血傷害後的再灌流方式，於灌流期先施以短暫且重複的缺血-灌流程序後，再恢復長期灌流。吾人以大鼠模式的研究發現，施與缺血後制約訓練可減少短暫全大腦缺血所造成的海馬迴 CA1 區域的細胞死亡。缺血後制約訓練的保護效應係透過減少粒線體中的 cytochrome C 釋放而達成 ，顯示缺血後制約訓練可能會減少重灌流傷害時粒線體的功能受損。

目次:

目的: 研究缺血後制約訓練對於大腦短暫缺血所引起的神經元細胞死亡的神經保護效果

背景:

Reperfusion injury is an important issue to deal with in treatment of ischemic diseases including myocardial ischemia and stroke. It has been shown that mitochondrial dysfunction is an early and major event happens in the reperfusion injury. Some strategies to ameliorate mitochondrial dysfunction and subsequent cell death have been proposed. Ischemic preconditioning is a protective strategy that some brief, minor injurious episodes of ischemia could reduce subsequent major ischemic insult. Such a strategy could be applied in some pretreatment preparations, including organ transplantations. However, the application of ischemic preconditioning to attenuate organs injury is limited in most ischemic diseases including stroke, for the occurrence of these diseases be not predicted.

Recently, it was reported that ischemic postconditioning, a modified reperfusion maneuver after major ischemia, could mitigate myocardial reperfusion insults. We would like to test the hypothesis that ischemic postconditoning reduce cell death after transient global cerebral ischemia, and the neuroprotective effect is related to mitochondria functions.

過程:

- 方法

1. Transient global cerebral ischemia (tGCI) model: We first established a model of transient global cerebral ischemia of brain on male Spraque-Dawley rats. Hypotension to below 30-35 mmHg was made on the rats by rapidly withdrawing the blood from jugular vein within 30 seconds. The bilateral common carotid arteries were then clamped by vascular clamps temporarily for 5 minutes. After 72 hours, delayed cell death of the CA1 neurons of hippocampus would happen. The tGCI rats would be our control group to evaluate the protective effects of ischemic postconditioning.
2. Ischemic postconditioning (iPoC): Ischemic postconditioning was made by repeating 3 cycles of short clamping-releasing on left common carotid artery immediately after the tGCI procedure. The end-point assessment includes cresyl violet staining, Fluoro-Jade C staining and Western blot analysis of CA1 neurons at the pre-determined time.
3. Assessment: The brain slices after treatment were stained with cresyl violet for histology. The CA1 region after treatment was harvested and the cytosolic portion of cells was extracted for Western blot analysis.

- 結果:

Histological analysis of hippocampal injury

Ischemic changes of cells in the CA1 pyramidal layer appears 3 days after tGCI. The cresyl violet stainingof coronal sections in the hippocampallCA1 subregion showed many dead cells with condensed, pyknotic nuclei (Fig.1). On the other hand, cells of CA1 subregion of the iPoc group showed well-defined cells with round nuclei.

Western blot analysis of cytochrome c release

In the cytosolic portion, the cytochrome c band was evident at 1, 6 and 24 hours after tGCI. As compared to the tGCI group, the cytochrome c immunoreactivity was weak at 1,6 and 24 hours after iPoC. The similar trend could be observed about the cleaved caspase 9 immunoreactivity expressed. The cleaved caspase 9 bands were less evident at 1, 6 and 24 hours after iPoc as compared with that of tGCI group.

Figure 1. Cresyl violet staining showed ischemic postconditioning reduce cell death in Ca1 region of hippocampus after tGCI

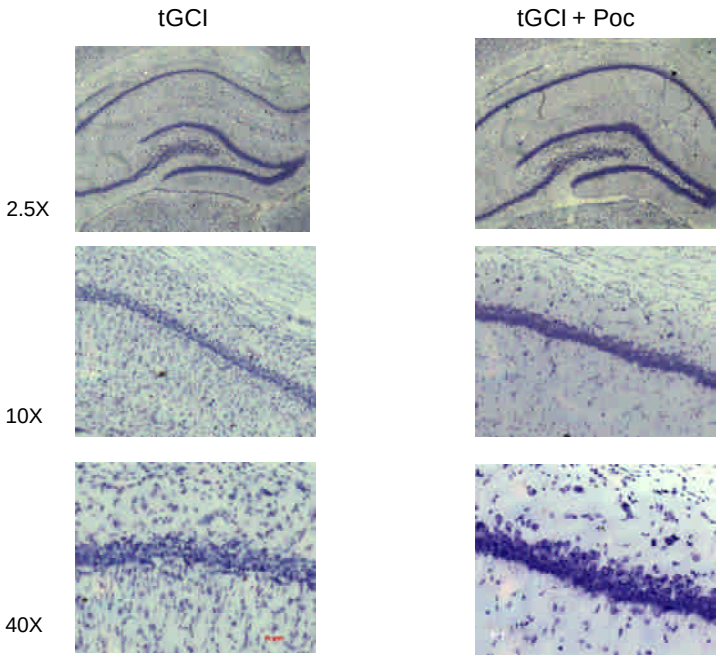
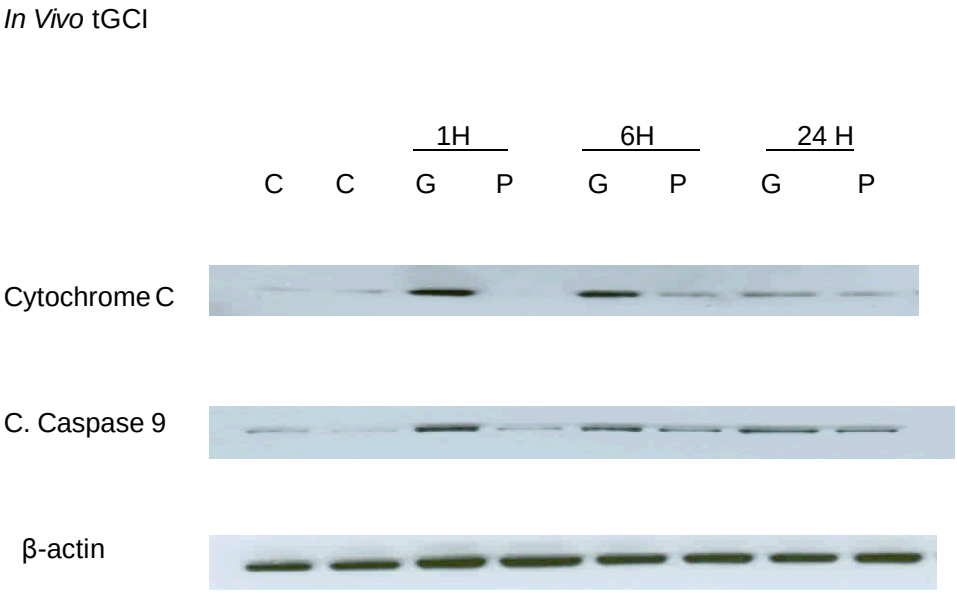


Figure 2.Western blot analysis showed iPoc reduced cytochrome c release from mitochondria after tGCI (C: control; G: GCI; P: iPoC)



心得:

吾人研究顯示缺血後制約訓練能減少短暫大腦缺血後的海馬迴 CA1 區域神經元的細胞死亡。缺血後制約訓練並減少粒線體在短暫大腦缺血後的. It cytochrome C 釋放，表示缺血後制約訓練的細胞保護效應可能透過減少粒線體受損而達成。

建議事項:

1. 缺血後制約訓練可以減少器官的缺血重灌流傷害。其保護機制值得進一步研究。
2. 粒線體不只是細胞的能源工廠，也是缺血重灌流傷害的重要標的。針對粒線體做爲治療目標可能開啓轉譯醫學的另一新領域。
3. 基礎研究需要投注時間與金錢。吾人建議出國的補助應適當延伸至 2 年