低溫對於大鼠星狀細胞所產生的藥理及毒理作用

PHARMACOLOGICAL AND TOXICOLOGICAL EFFECTS OF HYPOTHERMIA ON RAT ASTROCYTES

中文摘要

低溫對於缺氧狀態下的腦部組織具有神經保護作用，在臨床上也針對此特性加以應用，例如在體外心肺循環的情形下，適度降低體溫以增加對腦部組織的保護。但是有關低溫對於神經細胞的毒理及藥理研究仍然很少，本研究目的是要探討低溫對於神經細胞的毒理及藥理反應，以提供後續臨床應用的參考。本研究是以大鼠的星狀細胞（rat C6 glioma cell）為模式，將細胞低溫培養於 5 % 二氧化碳的培養箱中。本實驗將低溫分成輕度低溫（35 ℃）、中度低溫（32 ℃）、及深度低溫（26 ℃），將星狀細胞分別經過 2、4、6 小時的低溫暴露後，探討細胞存活率的變化，細胞大小的變化，並探討神經膠原酸化纖維蛋白質(glial fibrilary acidic protein) 及神經生長素(nerve growth factor)的 mRNA 數量表現的變化。實驗結果顯示，在輕度低溫及中度低溫培養下，細胞存活率並沒有明顯的變化；但是，在深度低溫下，細胞存活率會有意義地降低。同時隨著暴露時間的增長，細胞存活率會隨之減少。以共軛顯微鏡測量細胞的型態大小顯示，深度低溫暴露會使細胞形態大小呈現明顯的皺縮。利用 RT-PCR 的方法偵測神經生長素及神經膠原酸化纖維蛋白質的 mRNA 的變化，發現在深度低溫暴露 4 及 6 小時後，神經生長素 mRNA 的數值與對照組相比，有明顯的降低；而在神經膠原酸化纖維蛋白質 mRNA 的實驗中，在低溫暴露 2、4、6 小時後，神經膠原酸化纖維蛋白質 mRNA 數值都有明顯的增加。本研究的結果發現，在輕度及中度低溫培養下，對於星狀細胞沒有明顯的細胞毒性，但在深度低溫下，則有明顯的細胞毒性，同時深度低溫會使星狀細胞呈現細胞皺縮現象；並經由對於神經膠原酸化纖維蛋白質 mRNA 生成的增加，影響細胞結構的穩定，以及神經生長素 mRNA 生成量的減少，影響其他神經細胞的功能。

英文摘要

Hypothermia was a neuroprotective agent under ischemia brain. Clinical application of hypothermia treatment on neurosurgical and cardiovascular surgery are common. But basic study about the hypothermia effect on glial cell was rare. The aim of this study was to evaluate the effect of hypothermia on rat C6 glioma cell. Rat C6 glioma cells were cultured at 5 % CO2 incubator. The degree of hypothermia was defined as mild（35 ℃）, moderate（32 ℃）, and deep（26 ℃）. Rat C6 glioma cells were cultured at various degree of hypothermia for 2、4、 6 hours. We used trypan blue exclusion method and MTT assay to evaluate the cell viability. The cell size was determined by confocal microscopy. The expression of glial fibrilary acidic protein and nerve growth factor mRNA by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) analysis was test to evaluate the pharmacological effect of deep hypothermia. No direct cytotoxicity on C6 glioma cell at mild and moderate hypothermia was observed. Deep hypothermia decreased cell viability at 2、4、6 hours. Decrease in cell size during deep hypothermia treatment under confocal microscope was obvious at 2、4、6 hours. Increased expression of GFAP mRNA at 2、4、6 hours of deep hypothermia and decreased expression of NGF mRNA at 4、6 hours of deep hypothermia was also noted. We concluded that deep hypothermia not only had a direct cytotoxicity on rat C6 glioma cell but also had negative effect on the CNS via decrease the expression of nerve growth factor and increase the expression of glial fibrilary acidic protein.