行政院國家科學委員會補助專題研究計畫成果報告
神經保護藥物對粒線體功能異常時鼠腦病變的影響

計畫類別：■個別型計畫　□整合型計畫

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中文摘要

3-Nitropropionic acid (3-NP)為粒線體呼吸鏈複合體 II 的抑制劑，它可在鼠導致選擇性紋狀體病變, 如同人類的 Huntington 病。過去的許多研究包括我們的研究顯示，抑制興奮性氨基酸的釋放和施予 NMDA 受體的拮抗物，會減輕粒線體毒物引起的腦病變。最近的研究也顯示，Dichloroacetate (DCA)對心臟衰竭及腦缺血的鼠有保護作用。DCA 也被用於治療粒線體腦病變。為了更進一步了解 DCA 是否也可用於減輕 3-NP 所致的腦病變，及這些藥物對腦代謝物的影響，在本計畫中，我們利用磁共振影像(MRI)(T2 圖譜)和生物活體磁共振質譜(in vivo ^{1}H MRS)的變化來評估 DCA 對粒線體功能異常所引發腦病變的治療效果。我們以 3-NP 為實驗藥物，以迷你注射器包埋於二個月大的 Sprague-Dawley 株鼠腹部皮下，並以 MK-801(2mg/kg)和 DCA (100mg/kg)為治療藥物，比較治療的效果。我們先觀察慢性 3-NP 注射對鼠行為和腦病變的影響。然後觀察 DCA 對亞急性 3-NP 腦病變的影響。結果顯示慢性 3-NP 注射可使鼠產生肢體障礙類似 Huntington 病，如同亞急性注射。同時鼠腦紋狀體也會產生病變。生物活體磁共振質譜也顯示 NAA 下降, 表示神經元的死亡。於亞急性的動物模式中，DCA 的治療並不減輕 3-NP 對鼠腦紋狀體的傷害。DCA 的臨床運用需更進一步的深入探討其可行性。

關鍵詞：3-Nitropropionic acid, Huntington 病, dichloroacetate.

Abstract

Systemic injection of 3-nitropropionic acid (3-NP), an irreversible inhibitor of complex II in mitochondrial respiratory chain, induces selective striatal lesions in rats and non-human primates mimicking those in Huntington's disease. In recent studies, dichloroacetate (DCA) was shown to have protective effects in rat models of cerebral ischemia and myocardial dysfunction. However, its therapeutic effect on brain lesions induced by mitochondrial dysfunction is rarely investigated. In the past year, we established an in vivo animal model to evaluate the rat brain lesions in mitochondrial dysfuction. In the present study, we compared the therapeutic effect of DCA and MK-801 by in vivo animal model.

Two-month-old Sprague-Dawley rats were treated with 3-NP by continuous drug release from mini-pump, implanted subcutaneously. MK-801 (2mg/kg) and DCA (100mg/kg) were given in the same way. The rats were then evaluated by MRI (T2 maps) and in vivo ^{1}H-MRS at indicated time points. We first evaluated the effect of chronic 3-NP injection on rats, and then evaluated the effect of DCA on the striatal lesions induced by 5-day 3-NP injection. The results showed that chronic 3-NP injection produced behavioral change and selective striatal lesions on rats. MRS also showed the decline of NAA/Cr ratio, indicating the neuronal loss or dysfunction. The simultaneous application of DCA showed no attenuation of the striatal lesions. The present results suggest that the

application of DCA in brain lesions induced by mitochondrial inhibitors need further investigation.

Keywords: 3-Nitropropionic acid,
Huntington disease,
dichloroacetate.

緣由與目的

Neurodegenerative diseases have been found to be associated with mitochondrial dysfunction as in mitochondrial diseases in recent years. Systemic administration of 3-nitropropionic acid (3-NP), an irreversible inhibitor of complex II in mitochondrial respiratory chain, induces selective striatal lesions in rats and non-human primates mimicking those in Huntington's disease (Lee et al, 2000a and b). Several drugs have been shown to have neuroprotective effect in 3NP-treated rats, including glutamate release inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists. In recent years, dichloroacetate (DCA) was shown to have protective effect for cerebral ischemia in rats (Peeling et al., 1996), and was also used as metabolic therapy for myocardial ischemia and failure. DCA can stimulate the pyruvate dehydrogenase complex activity, and, therefore, can improve the recovery of cerebral lactate, acidosis and ATP following reperfusion in forebrain ischemia of rats. Because persistent lactic acidosis was supposed to be a factor contributing to the neuronal injury, decreasing cerebral lactic acidosis and enhancing cerebral metabolism may be one of the strategies in ameliorating brain injury related to elevated lactate, such as cerebral ischemia. DCA has also been implicated in the therapy of patients with MELAS and Leigh disease in recent years (DeStefano et al., 1995; Pavlakis et al., 1998; Saitoh et al., 1998), especially in Japan. However, there are limited in vivo researches about the application of DCA in neurodegenerative diseaseas. Therefore, further investigation of the therapeutic efficacy is highly needed.

方法

Two-month-old Sprague-Dawley rats were used. 3-NP was injected by mini-pump, implanted subcutaneously. MK-801 (2mg/kg) and DCA (100mg/kg) were used as therapeutic drugs. 3-NP-induced behavioral changes in the animals were recorded and graded according to the neurological scale described by Guyot et al. (1997). The 3-NP-induced brain lesions were evaluated by MRI (T2 maps) and in vivo proton MRS.

We first evaluated the effect of chronic 3-NP injection on the behavior and brain lesions, and then evaluated the effect of DCA and Mk-801 on the brain lesions induced by 5-day 3-NP injection.

Magnetic resonance measurements were performed on a 4.7 Tesla spectrometer (Bruker Biospec) with an active shielding gradient at 6.9 G/cm in 500 μsec. The rats were placed in a prone position with a custom-designed head-holder. A 20cm birdcage coil was used for RF excitation, and a 2cm-diameter surface coil placing directly over the head was used for signal receiving. After magnetic field optimization, a multi-slice multi-echo imaging was obtained with the following parameters: field of view = 5cm, 4 slices (2 mm thick with 1 mm gap), matrix = 256 x 128, TR = 4000msec, and initial TE = 20msec with an echo spacing of 20msec for 6 echoes. Data processing for T2 maps was performed with commercial image analysis software (MRVision Co. CA, U.S.A.).

The point-resolved spectroscopy (PRESS) sequence preceded by three consecutive CHESS pulses for water suppression was used to acquire the localized proton spectra over the striatum. Each CHESS pulse for water suppression was 15 msec in duration, and was followed by a spoiled gradient. CHESS pulses of 2msec duration for the 90 and 180 pulses were used. After manual adjustment of the transmitter and receiver, shimming of the region of interest and maximizing the suppression of water signal, localized spectral data was obtained with the following parameters: TR = 2000msec, TE = 136msec, scan no. = 256,

spectral width = 4000 Hz. The peaks for N-acetylaspartate (NAA), choline (Cho), creatine (Cr), succinate (Suc) and lactate (Lac) were recognized. The ratios of NAA/Cr, Suc/Cr, Cho/Cr and Lac/Cr were used for data analysis.

結果與討論

The present results showed that chronic application of 3-NP produced behavioral changes mimicking those in Huntington disease. There were also selective striatal lesions on rat brain with gradual decrease of NAA/Cr ratios over the striatal areas, indicating the neuronal loss or dysfunction. Elevated Suc/Cr and Lac/Cr ratios were also noted in chronic 3-NP injection.

Subacute 3-NP injection by mini-pump also produced behavioral changes and selective striatal lesions as shown in our previous studies (Lee et al, 2000a). Simultaneous application of DCA, however, did not abolish the behavioral changed and striatal lesions induced by subacute 3-NP injection. Compared with DCA, MK-801 showed prominent protective effect with attenuation of the striatal lesions induced by 3-NP as shown before (Lee et al, 2000a).

Although DCA had been shown to be effective in attenuation of ischemic cerebral lesions in rats (Peeling et al, 1996), it is not effective in alleviating the striatal lesions induced by 3-NP. Hypoxia and ischemia may suppress the activity of pyruvate dehydrogenase complex with accumulation of lactate. DCA can stimulate the pyruvate dehydrogenase complex activity, and, therefore, can improve the recovery of cerebral lactate, acidosis and ATP following reperfusion in forebrain ischemia of rats. In contrast, the accumulation of lactate in 3-NP-induced brain lesions results from the inhibition of respiratory chain complex II. Therefore, the stimulation of pyruvate dehydrogenase complex activity may not be effective in improving the mitochondrial function. Another possibility is that 3-NP also inhibit the Kreb's cycle and, therefore, affect the cell metabolism and survival.

DCA has been tried in several kinds of mitochondrial encephalopathy. Although DCA was shown to be effective in treatment of patients with Leigh disease and MELAS (DeStefano et al., 1995; Pavlakis et al., 1998; Saitoh et al., 1998), its effect on other mitochondrial encephalopathies needs further investigation to see whether different mechanisms of mitochondrial encephalopathies will lead to different response of the patients to the treatment with DCA.

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