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• 計畫中文名稱	花生油酸在新生兒缺血-缺氧性腦病變所扮演的角色
• 計畫英文名稱	The Role of Arachidonic Acid on the Neonatal Hypoxic-Ischemia Encephalopathy
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• 中文關鍵字	新生兒缺氧-缺血腦病；花生四烯酸；神經細胞培養；神經毒性；乳酸去氫？；NMDA 受體；腦脊髓液；麩胺酸鹽
• 英文關鍵字	Neonatal hypoxic-ischemia encephalopathy；Arachidonic acid；Neuronal cell culture；Neurotoxicity；Lactate dehydrogenase；N-methyl-D-aspartate receptor (NMDA receptor)；Cerebrospinal fluid (CSF)；Glutamate
• 中文摘要	缺氧-缺血引起的腦神經細胞壞死的致病機轉相當複雜。目前已知過度活化興奮性氨基酸受體的一種亞型受體 N-methyl-D-aspartate (NMDA) 受體占有關鍵性的角色。過度活化 NMDA 受體所產生的細胞毒性有部分是來自透過活化 Phospholipase A2 產生花生油酸釋放。而花生油酸可經由多種？的作用產生許多具細胞毒性的代謝物如 Leucotriene、Free radical 及 Prosta 等。在活體組織及細胞培養上,已證實高量的 NMDA 可產生極明顯的花生油酸及其代謝物,其情形相似於缺氧-缺血狀況下所得的結果。有可能是否花生油酸及其代謝物產生的量與 NMDA 與缺氧-缺血導致的腦神經細胞損害會成密切的相關平。若是如此,則偵測花生油酸及其代謝物的量可成爲缺氧-缺血下神經細胞受損的指數。最近,實驗證實花生油酸可經由突觸前抑制麩氨酸(Glutamate)的再吸收以及突觸後直接對 NMDA 受體的促進作用,來增強 NMDA 受體所產生直接突觸傳導訊息。然而,此花生油酸對 NMDA 受體的促進作用在 NMDA 與缺氧-缺血引起的腦神經細胞毒性中所扮演的角色仍未被驗證。由於確認花生油酸是缺氧-缺血腦神經細胞壞死的作用機轉之一,在臨床上有嘗試去偵測病人受到缺氧-缺血事件後血中或腦脊髓液裡花生油酸及其代謝物。但甚少具體的報告,尤其是針對新生兒極重要的一種腦神經病變,即新生兒窒息引起的新生兒缺氧-缺血腦病。過去對新生兒缺氧-缺血腦病變的研究,有一部份是針對如何來估計腦神經細胞受損狀況並估計其癒後神經後遺症。除了以臨床表現、腦波變化及影像分析來做測定外,測量脊髓液中的生化成分也是用來做爲一種指數,這其中並包括測定 Lactic dehydrogenace(LDH) Ceratinive kinare Hypoxanthine 等。倘若花生油酸及其代謝物的增加量能反應神經細胞壞死的程度,則其在脊髓液中的濃度可以用爲估計病人腦部受損狀況的生化指數。綜合而言,基於上述所列的論點,我們設計一個兩年連續性計畫,特針對下列三項問題進行深入探討:(1)在細胞培養上檢驗花生油酸是否本身會增進 NMDA 及缺氧-缺血所產生的腦神經細胞的毒性。(2)在細胞培養上檢驗花生油酸及其代謝物增加的量是否與 NMDA 及缺

	氧-缺血所產生的腦神經細胞的毒性程度成平行的關係。(3)在有窒息狀況的新生兒上,偵測其脊髓液中花生油酸及其代謝物的量是否可成爲估計腦神經細胞受損程度的指標。 以上三項實驗計畫,不僅是希望能在實驗室內多了解花生油酸及 NMDA 受體產生缺氧-缺血腦神經細胞壞死所扮演的角色,且進一步嘗試證明偵測花生油酸及其代謝物在脊髓液中的量有臨床上使用的價值。 我們發現花生油酸可以在較高濃度下增強引發鈣離子內流,但卻無法對 NMDA 引發的細胞毒性產生作用。然而,若細胞暴露在花生油酸二小時以上便會導致細胞死亡,而這種細胞死亡可以被 NDGA 一種花生油酸代謝酵素的抑制劑所抑制,花生油酸在高濃度下可增進細胞因缺氧缺糖下所產生的死亡。
• 英文摘要	Both arachidonic acid (AA), an important oxygenated polyunsaturated fatty acid in cell membrane, and N-methyl-D-aspartate (NMDA) receptor, one of glutamate subtype receptor, have been implicated in neonatal hypoxia-ischemia encephalopathy (HIE). In animal studies, overactivation of the NMDA receptor creates a calcium-sensitive neurotoxicity similar to that of hypoxia-ischemia insult. In both toxic conditions, there is consistent increase in the level of AA and its metabolites which paralleling well with the development of cellular damage. Conceivably, the toxic effect of NMDA is partly contributed by the activation of phospholipase A2 (PLA2). Activation of PLA2 results in the release of AA, which then metabolized by lipooxygenase, cyclooxygenase and free radical generating enzymes to produce substances toxic to neuronal cells. We had determined the neurotoxicity of AA on the primary cortical neuronal culture. AA concentration-dependently whether there is a positive correlation between the level of released AA and its metabolites and severity of neuronal damage is not well determined yet. Recent investigation further demonstrate that, not only activation of NMDA receptor could trigger the release of AA, but also the released AA could potentiate NMDA-mediated neurotransmission by a post-synaptic mechanism. It is interesting to know whether this potentiating effect of AA could affect the neurotoxicity induced by either NMDA or hypoxia-ischemia. While the presentation of AA and its metabolites in extracellular fluid of brain elicited by hypoxia-ischemia has been well determined in animals models, few of human studies has been reports. Increased leukotriene C4 and prostaglandin E2 in CSF was observed in adult patients with cerebral stroke. In asphyxiated newborn, increased serum level thromboxane and 6-keto-PGF1.alpha. were observed in severe cases. Inspiring from these studies and those of animal studies, we hypothesize that the level of AA and its metabolites in CSF could reflect the degree of brain damage in newborn developed HIE after the asphyxia insult. If so then such assessment may be of clinical use. Taking together, to further investigate the role of AA on the hypoxia-ischemia induced neurotoxicity in both of animals and humans we propose a two-year project in determining the following issues 1) To determine whether AA could potentiate the NMDA- and hypoxia/ischemia induced neurotoxicity in primary neuronal cell cultures 2) To determine whether the level of AA and its metabolites will reflect the cell damage induced by NMDA- and hypoxia-ischemia in primary cortical neuronal cultures 3) To determine whether the level of AA and its metabolites in CSF will be a biochemical index for assessing the brain damage in newborns of HIE. The result of the first year study showed that AA could potentiate NMDA-induced /sup 45/Ca/sup 2+/ accumulation and neuronal death. In addition, when incubating the cells with AA for a longer period, AA itself produced neuronal cells death. The potency of this toxic effect of AA is dependent on the age in vitro of the culture and incubation time with AA. We also established a HPLC assay in quantifying the level of AA in blood and CSF. The determining limit is between 1.mu.M to 1mM. The collection of human clinical samples are still underwent.