三氧化二砷抑制血小板凝集作用之機轉探討

Mechanisms involved in the antiplatelet activity of arsenic trioxide(As2O3)

中文摘要

三氧化二砷 (Arsenic trioxide, As2O3) 俗稱砒霜。在化學上爲昇華礦物。95 % 都以 As2O3 的化合物型態存在於大自然中,而且在中醫學上運用 As2O3 已有好幾千年的歷史，美國食品藥物管理局 (FDA) 於 2000 年 9 月正是批准三氧化二砷使用於治療急性前髓球性白血病,可說是從傳統中藥發展爲現代製劑的最佳範例。已知低劑量的 As2O3 可用於抑制腫瘤的轉移 (且具有引導 NB4 cell 進入 apoptosis 的狀態)，已被證實具有抗癌的效果。然而 As2O3 在血小板上的藥理學功效尚未明確，因此我們有意探討 As2O3 在血小板活化過程中，對於訊息傳遞方面的抑制作用。本研究在於更深入討論 As2O3 在血小板活化的過程中，對於訊息傳遞的抑制作用。研究結果顯示 As2O3 隨者濃度增加 (5-300 mM)，能有效抑制 collagen (1 mg/ml) 與 U46619 (1 mM) 以及 arachidonic acid (AA) (60 mM) 所引起的人類血小板凝集反應以及 ATP 釋放反應。As2O3 (15 and 25 mM) 亦可抑制 collagen (1 mg/ml) 所引起的細胞內鈣離子移動，以及 thromboxane A2 (TxA2) 的合成。此外，As2O3 (15 and 25 mM) 可以增加人類血小板內 cyclic AMP 的含量，但對於 cyclic GMP 的含量並沒有顯著增加；另一方面 As2O3 (15 and 25 mM)可以有效清除 collagen (1 mg/ml) 刺激產生的 hydroxyl radicals。As2O3 可抑制以及抑制 collagen (1 mg/ml) 所刺激的 47 kDa protein 磷酸化，且可抑制由 collagen (10 mg/ml) 所刺激的 p38 磷酸化，但是相對於 PDBu (150 nM) 對 47 kDa protein 磷酸化及 extracellular signal regulated kinases (ERKs) 的磷酸化沒有抑制。 PDBu (150 nM) 和 collagen (1 mg/ml)可誘發 protein kinase C 的活化，並且將 47 kDa protein 磷酸化， As2O3 (15 和 25 mM) 可有效抑制 47 kDa protein 磷酸化。

由結果證實，As2O3 抑制血小板活性的作用可能牽涉下列路徑：(一) As2O3 會增加血小板細胞內 cyclic AMP 的含量，並且誘發 VASP 磷酸化，卻不會增加 cyclic GMP 的含量。 (二) As2O3 利用其清除 hydroxyl radicals 的作用以及抑制 p38 磷酸化,影響 phospholipase A2-cyclooxygenase 路徑的反應，進一步抑制 TxA2 的生合成。

綜合以上結果，導致 As2O3 抑制血小板細胞內鈣離子的移動以及濃度的增加，最後因而抑制血小板的凝集反應。而此項作用代表著 As2O3 可以有效地應用在治療與血小板過度活化相關之疾病。

英文摘要

The general public name of Arsenic trioxide (As2O3) is “pi shuang”. 95% of As2O3

is a chemistry compound abounds in the Nature. Arsenic trioxide has been used in the traditional Chinese medicine for thousands of year. The modern era for the clinical use of arsenic was began in the September 2000, when the FDA of United States approved the application of arsenic trioxide in the treatment of acute premyelocyticleukemia. This is the best example to the traditional Chinese medicine become the western medicine. The As2O3 was demonstrsted the feasibility of low dose arsenic trioxide as an adjuvant drug in the treatment of solid tumor, especially in the inhibition of tumor metastasis. However, the pharmacological functions of As2O3 in platelets were not yet understood, we are interesting to investigat the inhibitory effect of As2O3 in cellular signal transduction (let the NB4 cell apoptosis) of platelet activation. In this study, As2O3 concentration-dependently (5-300 mM) inhibited collagen (1 mg/ml), U46619 (1 mM), and arachidonic acid (AA) (60 mM) induced human platelets aggregation and ATP-release reaction. In addition, As2O3 (15 and 25 mM) markedly inhibited intracellular Ca2+ mobilization, phosphoinositide and thromboxane A2 formation in loaded platelet stimulated by collagen (1 mg/ml). Furthermore, As2O3 (15 and 25 mM) significantly increased the formations of nitrate and cyclic AMP but not cyclic GMP in human platelets. Moreover, As2O3 (15 and 25 mM) obviously inhibited hydroxyl radicals family and p38 MAPK phosphorylation in human platelets stimulated by collagen, but not significantly inhibited ERKs phosphorylation. Rapid phosphorylation of a protein of MW 47,000 (P47), a marker of protein kinase C activation, was triggered by PDBu (150 nM) or collagen (1 mg/ml). This phosphorylation was inhibited by As2O3 (15 and 25 mM) in human platelet. In conclusion, our study suggested that the possible pathways of anti-platelet aggregation of As2O3 may involve in the following pathway：(1) As2O3 increases cyclic AMP formation and stimulats phosphorylation of VASP. (2) As2O3 significantly inhibited thromboxane A2 formation through inhibition of phospholipase A2-cyclooxygenase pathway, resulting in the inhibition of intracellular Ca2+ mobilization, finally inhibited platelet aggregation. Taken together, As2O3 may be use as an effective tool in treating pathological disorder associated with platelet hyperaggregability.