三氧化二砷對人類急性白血病動物模式之探討

Characterization of the effects of arsenic trioxide in an animal model of human acute myeloid leukemia

中文摘要

由三氧化二砷對於治療急性髓性白血病（M3）之病人是一有效的藥劑。在一些體外實驗中發現到此藥劑對於癌症治療的作用機轉的機制主要在於停止細胞週期、造成細胞凋亡及促進分化。

而在我們的實驗主要是研究三氧化二砷對於非-M3 的急性髓性白血病的治療效果。首先，先利用六至八週大的 NOD/SCID 老鼠建立一動物模型，以靜脈注射的方式將老鼠注射入 HL-60 的細胞株以及病人白血病細胞，注射四個星期後，抽出小鼠之骨髓細胞利用單株抗體（hCD45-FITC、hCD33-PE）來作觀察，並給予小鼠注射三氧化二砷作藥物治療（5ug/0.5ml/per day），在四至六週的療期後，發現注射三氧化二砷的實驗組與注射 PBS 的對照組，HL-60 細胞所佔之百分比並無明顯的差別。另外，進一步分析發現骨髓細胞的細胞週期發現大都停於 G0/G1 的階段中。而在體外培養的實驗中發現有與基質細胞直接接觸的白血病細胞比未與間質細胞直接接觸的白血病細胞所產生的自我凋亡細胞數目明顯較少。所以由此可得知，小鼠骨髓中之微環境會保護細胞避免產生自我凋亡。另外，由小鼠骨髓細胞發現細胞體內含有大量的麩胱甘（glutathion），因為體內含有轉移酶能利用體內的麩胱甘來將體內的過量的砷轉化進而代謝掉，也許這就是小鼠對於所治療的三氧化二砷沒有療效的原因之一。

由以上的結果可以知道三氧化二砷對於非急性髓性白血病（M3）並不能達到預期之療效，而造成這樣的結果有許多種原因，例如：小鼠體內的細胞停於細胞休息期（G0/G1 phase）中、小鼠體內的微環境保護白血病細胞避免三氧化二砷的毒殺、或是小鼠體內快速產生抗藥性所造成的結果。

英文摘要

Arsenic trioxide（ATO） is a novel agent for acute promylocytic leukemia (APL).In vitro studies have demonstrated that arsenic trioxide induces cell cycle arrest and apoptosis in multiple cancers.

We wanted to examine the effects of arsenic trioxide in non-APL acute myeloid leukemia by using a NOD/SCID mice animal model. HL-60 leukemia cell line （AML-M2） and primary cells （AML-M2）were injected into 6- to 8- week-old irradiated mice intravenously and after 4 weeks of injection, the presence of leukemic cells was verified by antibodies (hCD45-FITC、hCD33-PE). Leukemic mice were then treated with arsenic trioxide（5ug/0.5ml/per day）. After 4-6 weeks of treatment, the percentage of HL-60 cell in the bone marrow was not significantly different

between control and treatment groups. Analyses of bone marrow leukemic cell revealed a high percentage cells arrested in G0/G1 phase. In vitro culture of HL-60 cells with murine BM MS-5 stromal cells also showed cell cycle arrest at G0/G1 phase. As the cytotoxicity of ATO is dependent might upon cell cycle. So the bone marrow microenvironment protects the cells from arsenic trioxide-induced apoptosis, and makes the leukemic cell insensitive to arsenic trioxide. We also found that of bone marrow leukemic cell had a higher level of intracellular glutathion, which might be the reason why the leukemic cells rapidly developed resistance to ATO.

Our study shows that ATO is not effective in non-APL in vivo, possibly by multiple mechanisms: （1）marked cell-cycle arrest