酒粕中高度醣化最終產物抑制劑的分離

Isolation of novel inhibitor of advanced glycosylation products from Sake Vinasse

中文摘要

由於營養過剩及飲食習慣的改變，糖尿病的發生率正快速的增加中。據估計公元二千年全球有一千五百萬糖尿病患者，到公元二千年將遽增至二千二百萬人。糖尿病常伴隨白內障、視網膜病變、心血管病變、腎臟病變、神經病變等慢性併發症，且一旦發生都將成爲伴隨病患餘生的不可逆慢性病。不但病人本身、病人家屬痛苦不勘，所耗費的醫療成本更是社會經濟的一大負擔。如何找到可以減輕或防止糖尿病併發症的藥物是目前醫學界最重要的課題之一。然而葡萄糖是營養成分，高血糖爲何會引發這麼多嚴重的併發症一直是醫學研究最重要的課題。所幸最近的研究顯示不論是因爲胰島素缺乏的第一型糖尿病或胰島素訊息傳遞之第二型糖尿病，都會因爲葡萄糖無法運送至細胞內代謝，而造成程度不一的高血糖。血糖增高之後往往會和蛋白質的胺基經非酵糖尿病併發症、過度糖化最終產物、快速篩檢法、藥物開發素的 Maillard 反應而產生一系列的螢光產物，統稱爲”過度糖化最終產物” (Advanced Glycosylation End Products，簡稱 AGEs)。AGEs 能改變組織蛋白的結構和功能，是糖尿病併發症的主要病因。除了經由非酵素的 Maillard 反應之外，過高的血糖會利用高 Km 的 aldose reductase 催化而進行所謂的 polyol 代謝路徑。polyol 代謝路徑會消耗 NADPH，而影響內因性抗氧化素 glutathione 的合成，造成氧化的壓力。polyol 代謝路徑還會產出過多的三碳醣，並進一步反應成 methylglyoxal 等帶有二個 carbonyl group 的高反應性中間產物；Methylglyoxal 也可以和蛋白質作用而產生 AGEs。Pimegidine (aminoguanidine)，是一種 nucleophilic agent 可以透過其 hydrazine group (-NH-NH2)及 guanidino group -NH-C(=NH) 阻斷 Maillard 反應的 Amadori 產物爲無反應性的產物而不至於形成高反應性的 AGEs。過去數年 aminoguanidine 曾被證實可以有效的減輕或避免糖尿病大白鼠併發症的產生，雖然 aminoguanidine 因爲嚴重的副作用及基因毒性而無法通過第三期臨床試驗，但已引起世界各國開發 AGEs 抑制藥物的熱潮。我國固有的方劑和中草藥中有許多長久以來被用於治療三多症的天然藥物，其中很多天然藥物都已被純化或半純化。可惜至今國內尙未開發針對抑制 AGEs 形成、或 AGEs 降解的藥物篩選法。現在我們研發能阻斷高反應性 dicarbonyl 基團及能抑制後 Amadori 步驟 (post-Amadoristage) 之藥物的高通量篩檢法，並用以篩選中草藥，期望能發現可以阻度 AGEs 形成之藥物，並以其結構用爲藥物開發之基礎。另外，我們從酒粕分離出的粗萃物，已經證實對於 amadori 產物的形成有抑制的效果，進一步期望能夠純化出單一結構的化合物，以利日後研究治療糖尿病併發症藥物的發展。

英文摘要

Due to over-nutrition and changes of food style, the occurrence rate of diabetes mellitus has been dramatically increased. It is estimated that the numbers of people with diabetes will be increased from 15.1 millions in year 2000 to 22.4 million in 2010, which stands for a 46 % increase. Diabetes is often associated with a variety of severe chronic complications, including cataract, diabetic retinopathy, cardiovascular disorders, diabetic nephropathy, and diabetic neuropathy. These chronic complications are mostly irreversible and the burden of medical care has become an important social economic issue. To develop drugs that can effectively alleviate or prevent diabetic complication in a timely manner is an important and difficult challenge. One of the major obstacle in drug development is partly because the exact mechanisms of hyperglycemia-induced complications are not clear. Recent evidence revealed that advanced glycosylation end products (AGEs) play a central role in mediating the diabetic sequelae. AGEs can be formed by nonenzymatic "Mallard reaction" of the caronyl group of glucose and the amino group of proteins, or by polyol pathway. Under hyperglycemic conditions, glucose can be converted to sorbitol by high Km aldose reductase, an enzyme used NADPH as cofactor. Reduction of NADPH will hamper the formation of glutathione, am endogenous antioxidant. Sorbitol can be further converted to fructose and triose by the polyol pathway and give rise to generation of methyl glyoxal. Methylglyoxal is characterized of its highly reactive dicarbonyl group, which can instantly react with protein to form AGEs. Pimegidine (aminoguanidine) is a nucleophilic agent owing reactive hydrazine group (-NH-NH2) and guanidino group -NH-C(=NH). These reaction centers can block the reactive carbonyl group or Amadori product and prevent AGEs formation. In the past few years, Pimegidine has been shown to alleviate diabetic nephropathy in diabetic rat model. Although Pimegidine did not pass the phase II clinical trial due to its severe adverse effects and genome toxicity, these therapeutic targets has encourage many cutting edge researches. Many Chinese herbal drugs have been used to treat diabetes for thousand years. The purified or partially purified compounds from natural products possess diversified structural features and are excellent compound library for anti-diabetic drug development. Our laboratory has been working on AGEs for years and has generated a lot of important research materials and used them to develop many assay protocols including competitive ELISA and automatic AGEs measurement protocol. We have developd that a series of high throughput screening assay based on the mechanism of AGEs formation. We hope these high throughput screen assay will enhance the identification of lead compounds that are inhibit or break AGEs formation.