Isosteviol 對心臟血管的藥理作用之研究

The cardiovascular effects of Isosteviol

中文摘要

高血壓為導致心臟衰竭(heart failure)之主要病因，高血壓若沒予以適切的治療，則會造成左心室肥大而致心室舒張功能不良；高血壓亦為導致心肌梗塞的危險因子，一旦發生心肌梗塞則進而造成心室收縮及舒張功能都不良。目前認為，心臟衰竭是造成心臟血管疾病死亡率昇高的主要原因，在美國，65 歲以上人口住院第一位即為心臟衰竭。雖然，高血壓的機轉尚有很多未明的地方，若能及早診斷及治療，則可降低這項心臟血管疾病的併發症。因此，研發新的治療藥物是值得努力的工作。

先前的研究已知，甜菊(stevioside)在高血壓老鼠可有效的降低血壓，而且，它在日本及巴西當作代糖已有ニ十年之久，並無任何不良反應的報告。Isosteviol 為 stevioside 的衍生物，本研究旨在探討 isosteviol的效果及其機轉。

為進一步探討 isosteviol除了有舒張血管的功能以外，是否亦有抗心室肥大(ventricular hypertrophy)的功效？本研究以內皮素(endothelin-1,ET-1)當刺激劑(stimulant)與新生 1-2 天的小鼠心室肌細胞一起培養，看到內皮素可使細胞變肥大，亦可偵測得β-肌球蛋白重鏈促進子活性(β-myosin heavy chain promoter activity)的昇高。若先給予 isosteviol則有效地預防心室肌細胞變肥大，並且，亦可抑制 ET-1 所引起的β-myosin heavy chain promoter activity 的昇高。另外，isosteviol亦可明顯降低 ET-1 刺激細胞內所昇高[3H]-leucine incorporation 的量，而且，isosteviol降低 ET-1 刺激蛋白質合成量的效力與其使用量成正比(dose-dependant)。這項結果顯示 isosteviol對心肌細胞具有抗肥大(anti-hypertrophy)的效力。

想進一步更深入探討 isosteviol的抗心室細胞肥大機轉，參照既有的報告，在 ET-1 所引致的心臟血管病變會激發 extracellular signal regulated kinase (ERK) pathway，而且，活性氧化物(ROS)扮演十分重要的調控角色。於是，探討 isosteviol對 ET-1 在細胞內所產生的 ROS 及 ERK pathway 是否亦有調節或影響的作用。結果顯示 isosteviol能明顯且有效地預防 ET-1 對心室肌細胞刺激引起 NADPH oxidase activity 及 dichlorofluorescein (DCF) fluorescence intensity 的昇高，isosteviol具有降低細胞內產生 ROS 的效力。並且 isosteviol可抑制 ET-1 所激發 ERK1/2 的 phosphorylation；顯示 isosteviol經由調控(降低)ROS 的產生來減輕 ET-1 刺激心室肌細胞β myosin heavy chain promoter的 activity 及降低蛋白質的合成而達到心肌細胞不易肥大的效果。

總而言之，本實驗的結果，觀察到 isosteviol亦具有抗心室肌細胞肥大的效力，其作用機轉應為降低細胞內 ROS 的量，並可抑制 ERK1/2 的

phosphorylation 及β-myosin heavy chain promoter activity；顯示isosteviol具有心臟保護的作用(cardio-protective effect)。若配合已知的血管舒張作用，isosteviol既能降血壓，又具抗心肌肥大的效果，可算是一種理想的心血管疾病的良物。因此，它在臨床的應用，深具潛力。

英文摘要

Hypertension plays a key role in the formation of heart failure and it has been identified as the main precursor of left ventricular hypertrophy. Hypertension and myocardial infarction are the two risk factors for heart failure that is mentioned as one important factor for cardiovascular morbidity and mortality in recent. It has been established that early diagnosis and effective treatment can prevent the cardiac complications.

Isosteviol is one of the derivatives of stevioside, a constituent of Stevia rebaudiana, which is widely used as the noncaloric sugar substitute in Japan and Brazil for more than 20 years without any untoward effect. Stevioside had been identified to show anti-hypertensive effect and isosteviol was found to produce vasodilatation. In the present study, anti-hypertrophic effect and the possible mechanism(s) of isosteviol were investigated.

In the neonatal cardiomyocytes, endotehlin-1 (ET-1) caused ventricular hypertrophy characterizing by the increased expression of cardiac β-myosin heavy chain promoter that deteriorated the contractile function of ventricular cell. These changes were dose-dependently inhibited by the pre-treatment of isosteviol (0.1-10 μM). Also, ET-1 (10 nM)-induced the increase of in protein synthesis using [3H]-leucine incorporation in cardiomyocyte as indicator and increased the cell size apparently in morphology. Isosteviol inhibited this ET-1-induced cardiac hypertrophy and lowered theβ-myosin heavy chain promoter activity. The anti-hypertrophic action of isosteviol can thus be considered.

Then, the effects of isosteviol on ET-1 (10 nM)-induced reactive oxygen species (ROS) formation and the ERK1/2 signaling pathway were investigated. Cardiomyocytes exposure to ET-1 (10 nM) for 30 minutes immediately increased the NADPH oxidase activity and ROS formation indicated by the increase of 2’7’-dichlorofluorescein (DCF) fluorescence. Also, ET-1 rapidly activated the phosphorylation of ERK1/2. However, in the presence of isosteviol (0.1-10 μM), cardiomyocytes showed significantly decrease of NADPH oxidase activity, ROS formation and ERK1/2 phosphorylation, respectively. Moreover, isosteviol modulated ET-1-increased protein synthesis or β-myosin heavy chain promoter activity via an attenuation of ROS generation. Therefore, ROS is mediated in ET-1-activated ERK1/2 signaling of cardiomyocytes. Pretreatment with isosteviol could attenuate the

generation of ROS to reduce this stress mediated signaling for cardio-protective effect.

In conclusion, isosteviol is found to show anti-hypertrophic effect in the in vitro experiments. In addition to the vasodilatation in isolated aorta of rats, isosteviol also possessed anti-hypertrophy effect on ET-1 induced hypertrophy in neonatal cardiomyocytes. Thus, isosteviol has a potential to develop as therapeutic agent for clinical application in the future.