行政院國家科學委員會補助專題研究計畫成果報告

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※ Captopril 及 Iosartan 對大鼠原位肝細胞癌血管新生之影響 ※

※ **The effect of Captopril and Iosartan on Angiogenesis** ※

※ **of Rat Orthotopic Hepatocellular Carcinoma** ※

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執行期間：88 年　8 月　1 日至　89 年　7 月　31 日

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一、中文摘要

在許多腫瘤的研究中發現，血管新生現象和腫瘤的形成及進展密不可分，最近也有許多血管新生的研究特別重視它和癌症細胞轉移的關係，因而抑制腫瘤血管新生對於腫瘤的治療是一個新的方向。目前對於肝細胞癌血管新生的研究報告大多在於分析腫瘤細胞株在動物皮下組織分泌血管生成因子的能力或利用人類肝細胞腫瘤組織回溯性地研究血管生成因子在腫瘤形成過程中所扮演的角色，而針對原位性肝細胞癌血管新生抑制因子前溯性的研究則付之闕如。

Captopril 為血管增壓素轉換脢抑制劑，可以減少血管增壓素 II 的形成，常用於高血壓的控制，目前有研究發現其可以抑制血管內皮細胞 DNA 的合成進而抑制血管新生的現象，然而其血管新生抑制機制是否經由抑制血管增壓素轉換脢而作用，或是經由另一新的途徑則值得研究。Iosartan 也用於高血壓的調控，為血管增壓素 II 受器的拮抗劑，可以減少血管增壓素 II 的作用，此藥物是否如同 Captopril 有新生血管抑制作用，間接證明血管增壓素 II 是否為血管新生途徑之一也是值得研究的。

本實驗的特性在於其前瞻性，in vivo study，利用大鼠肝細胞癌細胞株 GP7TB，先注入老鼠的皮下約二三週等到腫瘤形成，再將腫瘤切除並切成約一立方毫米之腫瘤塊，並將此小腫瘤塊利用手術方式植入同種老鼠的肝臟中，做出活體原位肝細胞腫瘤的動物模式，並將此老鼠分成對照組、Captopril (CA)組（餵食 60 mg/kg/day 之 Captopril）及 Iosartan (I)組（餵食 20 mg/kg/day 之 Iosartan）三組。在植入腫瘤後十四及二十一天取出肝臟，測量腫瘤大小、重量，並且分別定量血管內皮細胞生長因子(vascular endothelial growth factor)、鹼性纖維母細胞生長因子(Basic fibroblast growth factor)及其 mRNA expression 能力，以檢驗 Captopril 以及 Iosartan 對於原位肝細胞癌血管生成抑制作用的程度以及差別，並探討其中機制的異同。

關鍵詞:大鼠肝細胞癌，血管新生，血管內皮細胞生長因子、鹼性纖維母細胞生長因子，微小血管密度，Iosartan，Captopril

Abstract

The assessment of angiogenesis provides a promising horizon in the pathogenesis of a variety of cancer diseases. Recent studies of angiogenesis demonstrated an important role of angiogenesis in cancer growth and metastasis. In the mean time, inhibition of angiogenesis might provide a new approach in cancer treatment. Reports concerning angiogenesis of hepatocellular carcinoma were restricted to either secretion function of angiogenic factors in cell lines or to histochemical studies on human hepatocellular carcinoma tissue retrospectively at present. Unfortunately, a prospective study of anti-angiogenesis in an orthotopic hepatocellular carcinoma in rat is

not present.

Captopril is an inhibitor of angiotensin converting enzyme, which can reduce the production of angiotensin II, and is widely used clinically in the management of hypertesive disease. Inhibition of DNA production in endothelial cells by captopril resulted in decreasing of angiogenesis had been reported recently. But further researches are demanded to study the mechanism. Iosartan, a new developed drug for hypertension treatment, is an antagonist of angiotensin II subtype I receptor with competitive inhibition of angiotensin II. It's worth to understand the effect of drug in order to clarify the role of angiotensin II in angiogenesis.

In this study, we use the chemical carcinogen transformed hepatic epithelial cancer cell line, GP7TB to establish an orthotopic hepatocellular carcinoma model for in vivo study. GP7TB cell suspension is inoculated subcutaneous in the posterior flanks of the rats. Two to three weeks later, tumor formed at subcutaneous region will be excised and cut into uniform fragments of about 1 mm^3. Then the fragment is transplanted into the liver of syngeneic Fischer 334 rats. The rats will be divided into 3 groups. In Control (C) group, only normal saline was given, In Captopril (CA) group, 60 mg/kg/day of Captopril will be given. In Iosartan (I) group, 20 mg/kg/day of Iosartan will be given. After 14 and 21 days, the rats will be sacrificed and the size, weight of the tumor will be recorded. In addition, quantification of angiogenic factors including vascular epithelial growth factor, and basic fibroblast growth factor as well as ability of mRNA expression will be done at the same time. The differences of anti-angiogenic ability and mechanism between Captopril and Iosartan will be discussed.

Keywords:
Rat hepatocellular carcinoma, Angiogenesis, Vascular endothelial growth factor, Basic fibroblast growth factor, microvessel density, Captopril, Iosartan

二、緣由與目的

肝細胞癌(Hepatocellular carcinoma)**為國內惡性腫瘤死亡之主要疾病，根據統計，台灣肝細胞癌的發生率於男性為每十萬人有二十五人，而女性每十萬人中也有十人，而每年因肝細胞癌死亡的病例高達五千人。雖然對於肝細胞癌的治療方式有外科切除、化學治療等方式，但仍然無法避免腫瘤復發的可能性，因此對於腫瘤本身分子生物學方面的研究就顯得更加重要。血管新生現象**(angiogenesis)**除了在正常組織的生長、傷口癒合等過程中佔有重要的地位，在許多炎性反應包括關節炎以及許多腫瘤的形成、轉移也不可或缺。近年來報導的血管新生因子包括** TGFβ[1]**、**TNFα[2]**、以及** Angiogenin[3]**，但是其對於血管內皮細胞的作用較少，至於鹼性纖維母細胞因子**(Basic fibroblast growth factor)**則可作用造成血管內皮細胞的增生** [4]**。此外更發現血管內皮細胞生長因子**(Vascular endothelial growth factor)**不但可以促進血管內皮細胞的增生** [5,6] **且可促使雞絨毛尿囊膜及大鼠角膜的血管新生現象** [7,8,9]**。最近對於肝細胞癌的血管新生之研究極為積極，目前已知鹼性纖維母細胞因子在正常人及慢性肝炎病人中並不增高，但部分肝硬化病人及大部分肝細胞癌病人則有增高現象** [10]**，且和肝細胞癌的侵襲性有關** [11]**。可惜的是目前對於肝細胞癌血管新生的研究報告大多利用腫瘤細胞株**(Cell line)**在培養皿**(In vitro)**或在動物皮下組織**(In vivo)**分析分泌血管生成因子的能力，或利用人類肝細胞腫瘤組織切片回溯性**(retrospective)**地研究血管生成因子在腫瘤形成過程中所扮演的角色，而仍無法做到原位性肝細胞腫瘤血管新生抑制劑動物模式的研究，這也是肝細胞癌血管新生研究的最大缺點。**

Captopril(D-3-metrcapto-2-methylpropanoyl-L-proline)**為血管增壓素轉換脢競爭性的抑制劑**(competitive inhibitor of angiotensin- converting enzyme, ACE)**，可以減少血管增壓素** II **的合成，目前全世界有五百到一千萬人使用於高血壓的控制及心衰竭的治療** [16, 17,18]**，此藥物尚可減緩**

關節炎[19]，改善糖尿病引起的視網膜病變[20]並減少下肢靜脈血拴的發生率[21]。目前有研究發現隨著腫瘤的成長，血管增壓素受器及血管增壓素轉換胺會逐漸出現[22]。此外 Captopril 可以抑制血管內皮細胞 DNA 的合成進而抑制血管新生的現象[23]，並抑制大鼠角膜血管新生現象[24]及治療卡波西肉瘤[25]。然而其對於肝細胞腫瘤的作用以及血管新生抑制機制是否經由抑制血管增壓素轉換胺或經由另一新的途徑則值得進一步的研究。

Iosartan 為血管增壓素 II 受器的拮抗劑 (antagonist of angiotensin II subtype I receptor)，目前也被用於高血壓的調控[26, 27]，可以減少血管增壓素 II 的作用，藉由探討此藥物是否有新生血管抑制作用，並比較與 Captopril 的異同，有助於探討血管增壓素 II 是否為血管新生之所需途徑，也可間接證實 Captopril 的血管抑制作用是否經由完全不同的途徑也是值得研究的。

設計此研究計畫的目的是:

1. 建立前瞻性大鼠活體原位肝細胞癌之實驗模式
2. 研究活體肝細胞癌腫瘤中血管內皮新生因子、鹼性纖維母細胞生長因子與腫瘤間的關係
3. 評估 Captopril 對於肝細胞腫瘤血管新生的抑制作用，並探討其對於 VEGF, B-FGF 的表現(expression)之影響
4. 評估 Iosartan 抑制肝細胞腫瘤血管新生的效果，並探討在 Iosartan 存在下 VEGF 及 B-FGF 的表現(expression)有何影響
5. 經由 Captopril 以及 Iosartan 對肝細胞癌血管新生抑制作用的異同可探討 Angiotensin system 在肝細胞癌血管新生中所扮演的角色

三、結果與討論

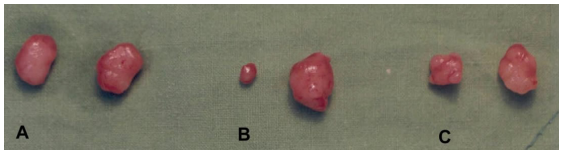

Fig. 1 The tumor size at fourteenth day in Control group (A), Iosartan Group (B) and Captopril group (C).

Fourteen days after tumor implantation, the tumor was weighted 0.456±0.034 g in C group, 0.381±0.216 g in I group and 0.262±0.047 g in CA group (Fig. 1). Twenty-one days after tumor implantation, the tumor weighted 0.751±0.157 g in C group, 0.700±0.245 g in I group and 0.371±0.088 g in CA group (Fig. 2).

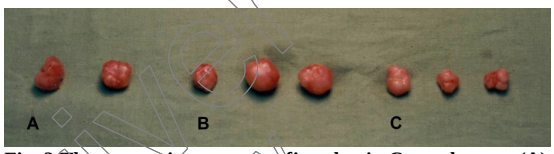

Fig. 2 The tumor size at twenty-first day in Control group (A), Iosartan Group (B) and Captopril group (C).

The confidence interval for the weight of tumor at fourteenth day was between 0.427 and 0.486 in C group, between 0.192 and 0.570 in I group, and between 0.221 and 0.304 in CA group. The confident interval for the weight of the tumor at twenty-first day was between 0.614 and 0.889 in C group, between 0.485 and 0.914 in I group, and between 0.294 and 0.448 in CA group. There was no significant difference between C group and I group, or between I group and CA group at fourteenth day. But the difference between C group and CA group was statistically significant ($p<0.05$). Although the difference between C group and I group was statistically insignificant, the differences between C group and CA group and between I group and CA group were statistically significant at twenty-first day.

In other way, in C group, the tumor grew significantly from fourteenth to twenty-first day. But the tumor growth was no significant from fourteenth to twenty-first day in I and CA groups. It indicated that without medication, the tumor growth was fast at the first fourteen days and became

much bigger seven days later. After Iosartan treatment, the tumor size varied considerably. Even though there was no difference as compared with C group, the tumor did not growth significantly seven days after the fourteenth day. So, inhibition of the tumor growth might happened in I group after 14 days of therapy. It means that as the therapy of Iosartan continued, the competitional inhibition of angiontensin II receptor might play certain role in inhibition of angiogenesis resulted in retardation of the tumor growth. But for CA group, the inhibition of tumor growth occurred not only for the initial fourteen days but it persisted at twenty-one days' therapy. In addition to decrease the production of angiotensin II, Captopril might use other pathway to slow down the tumor growth not only at the early but also late stage in tumor growth.

In conclusion, it is indicated that in addition to be an antagonist of angiotensin II, Captopril can diminish the growth of hepatocellular carcinoma through other pathway that was not discovered in the past. Even though the effect of Iosartan is obscure, it still offered some influences on the growth of hepatocellular carcinoma.

四、計畫成果自評

We have established an animal model in prospective study on hepatocellular carcinoma. Actually, it is also not easy to establish an in vivo animal model for study of the relationship between tumor angiogenesis and orthotopic hepatocellular carcinoma. In this study, it took much time to produce the tumor block in the back of the rat and introduce tumor block into the liver of another rats because type of hepatocellular carcinoma cell line, pH value, generations of tumor cell line, age of rats, et al. might made the growth of tumor retarded or even impossible.

Comparison of incidence of hepatocellular carcinoma in patients receiving Captopril and Iosartan for their hypertensive disease and that of the general population is important before the conclusion is made.

The study concerning about the expression of VEGF, β-FGF and their associated mRNA is still undergoing, and these results will make the conclusions more satisfactory.

Although in this experience it indicated that the Captopril have inhibitory effect on the growth of hepatocellular carcinoma in this in vivo animal model, further studies concerning about the mechanism of those medications are necessary in the near future.

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