銖 188 照射處理對行氣球擴張術冠狀動脈之基因表現的探討

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中 文 摘 要

研究背景：心血管疾病是台灣十大死因之第三位，過去數十年來也是許多西方國家主要的死因之一，動脈粥狀硬化是心血管疾病主要病因。氣球擴張手術可以有效擴張阻塞的冠狀動脈，已經發展成主要治療心血管疾病的方式。但是仍有 30% - 50% 接受氣球擴張手術的病人，半年內會有血管再狹窄的現象。血管平滑肌細胞增殖是氣球擴張手術後血管再狹窄的主要病理過程。即使有血管支架置入的病人，仍有 20% - 30% 患者在半年內會發生血管再狹窄的現象。放射線治療是目前醫學上用來治療惡性腫瘤的方法，此也可應用於血管再狹窄的預防，本研究利用反轉錄聚合酶連續反應技術 (RT-PCR) 及 cDNA 微陣列晶片，欲探討氣球擴張手術造成血管再阻塞和放射線處理對基因表現的影響，以便於日後更深入去探討血管在狹窄的治療分子機轉，並能應用到臨床上降低氣球擴張手術後冠狀動脈再狹窄的現象。

方法與結果：本實驗利用豬隻 （n = 24） 冠狀動脈狹窄模式。氣球擴張手術和放射線照射豬冠狀動脈的左前降支 (LAD)、左迴旋支 (LCX) 或右冠狀動脈 (RCA)，共有 72 條冠狀動脈。激態銖-188 放射性同位素，會放射出 β 粒子 (β_{max} = 2.1 MeV)，其半衰期為 16.9 小時。配合氣球擴張術的進行，液態銖-188 可置入血管氣球內以照射血管壁，此可達到均勻分佈血管壁照射的效果，並有效防止冠狀動脈再狹窄之發生。

實驗一為冠狀動脈再狹窄之病理觀察實驗，實驗分成五組，其分別為 PTCA only、Re-188 only、PTCA/Re、Re/PTCA、control。在手術處理後六週，犧牲動物並取出冠狀

動脈，先將取出之冠狀動脈以灌流方式固定，接著再進行蘇木精 - 伊紅染色 (H&E staining)，以觀察這些冠狀動脈的病理型態變化。control 組冠狀動脈內腔的面積 2250 ± 300 μm^2，然而只有進行氣球擴張手術的面積是 480 ± 150 μm^2，PTCA/Re 與 Re/PTCA 組的冠狀動脈內腔的面積皆比只有進行氣球擴張手術的面積大，其內腔面積分別為 1100 ± 200 μm^2 和 1300 ± 180 μm^2 ($p < 0.05$ vs. PTCA only 組)，結果顯示不管放射線照射處理先後都可增加氣球擴張手術導致內腔面積減小。另外，經過 20 Gy 的放射線照射與 14 Gy 放射線照射之比較，都可以顯著增加內腔面積和大量降低新生內膜組織形成。

實驗二為利用 RT-PCR 來探討豬隻接受氣球擴張手術和放射線治療 (20 Gy) 後的第一天，黏附分子的基因表現。ICAM-1 的基因表現量在 PTCA only、PTCA/Re (20 Gy) 及 Re (20 Gy)/PTCA 組皆有顯著的增加 ($p < 0.001$)。VCAM-1 的基因表現量在 PTCA only、Re-188 (20 Gy)only 及 Re (20 Gy)/PTCA 組皆有顯著的下降 ($p < 0.001$)；VCAM-1 的基因表現量在 PTCA/Re (20 Gy) 組有顯著的下降 ($p < 0.001$)。

實驗三為利用微陣列晶片方式來探討豬隻接受氣球擴張手術和放射線治療 (20 Gy) 後的第一天，基因表現的差異。在高表現基因而言，篩選出在 PTCA only 組高表現量的基因 444 個、只有進行 Re-188 (20) 照射組有 246 個、PTCA/Re (20) 組有 429 個、Re (20)/PTCA 組 234 個；而低表現的基因而言，依以上處理的組別分別有 918、612、707 及 653 個低表現量的基因。不論這些高低表現量基因為何，可利用 RT-PCR 和北方式點墨法等技術，進一步瞭解這些基因在血管再狹窄疾病的手術治療中所扮演的角色。

實驗四為體外試驗，利用人類平滑肌細胞株接受 2 與 20 Gy 的放射線照射，藉由 RT-PCR 探討 NF-κB 在照射後的第一、二及三天的基因表現量，實驗結果顯示：照射 2 Gy 後的第一天 NF-κB 的基因表現量較控制組表現量低四倍，然而第二、三天 NF-κB 的基因表現量增加，和未進行放射線照射的細胞沒有顯著差異。然而，經 20 Gy 放射線照射細胞，三天內 NF-κB 表現量都沒有和未照射的細胞有顯著差異。

結論：我們利用微陣列晶片的方法提供了一些表現基因初步篩選的結果，作為之後更進一步研究的依據。欲探討我們有興趣的基因時，可以利用北方式點墨法或 RT-PCR 實驗技術，進一步瞭解這些基因在血管再狹窄疾病中所扮演的角色。根據微陣列晶片的方法得到基因表現量的不同，幫助我們更清楚地瞭解氣球擴張手術和放射線治療與血管再狹窄疾病中，相關分子的作用機制。

Studying Gene Expression of Angioplastic Coronary Arteries

Treated with Rhenium-188 Irradiation

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Abstract

Background: Cardiovascular disease (CVD) has been the third cause of death in Taiwan. It remains the top cause of death in most western countries over past several decades. Atherosclerosis, one of the CVDs, is the main cause of coronary heart disease. One of the surgical treatments, percutaneous transluminal coronary angioplasty (PTCA), is to treat the stenosis of coronary artery and has been used to the primary method. However, 30% - 50% of patients after balloon angioplasty have renarrowing of vessels, called restenosis. One of the mechanisms is vascular smooth muscle cells proliferation. Besides, it had been the evidence that 20 - 30% of patients who had vessel stent implantation developed in-stent restenosis over a period of 6 months. Therefore, radiation therapy used to cure tumor is another method proven to effectively reduce in-stent restenosis. In this study, we aimed to study gene expression of coronary arteries after treatments of angioplasty and/or brachytherapy using the methods of reverse transcriptase polymerase chain reaction (RT-PCR) and cDNA microarray. We investigate the effect of gene expression in restenosis after the treatment of PTCA and radiation. It will be useful to study molecular mechanism of restenosis in detail. Besides, more uderstanding of mechanism can be an appliance for clinical trails in order to decrease a rate of restenosis after treatment of PTCA.

Methods and Results: Twenty-four pigs were used in porcine coronary arterial model. Seventy-two coronary arteries, including left anterior descending (LAD), left circumflex (LCX); and/or right coronary artery (RCA), were treated with angioplasty and/or brachytherapy. Rhenium-188 (^{188}Re) was used as a radioisotope and emitted β radiation, of which maximal energy is 2.12 MeV and half-life is 16.9 h. While accompanying the treatment of PTCA, ^{188}Re liquid filled balloon can complicate the achievement of a homogeneous dose distribution without centering of the irradiation source and effectively prevent the coronary restenosis.

The first experiment was the histopathological observation of coronary arterial restenosis. There were five groups, including angioplasty only, brachytherapy only, angioplasty prior to brachtherapy, angioplasty after brachytherapy, and control (PTCA only, Re-188 only, PTCA/Re, Re/PTCA, and control, respectively). Six weeks after the surgical procedures, the animals were sacrificed, and the treated coronary arteries were perfusion - fixed and stained. Lumen and neointimal areas were observed in histopathology and analyzed computer-aided histomorphometry. In the sham control group, the lumen area was 2250 ± 300 μm^2. However, the lumen area was 480 ± 150 μm^2 in the PTCA group. The lumen area of coronary arteries in response to angioplasty prior to and after brachytherapy was greater than that in the PTCA treatment alone. The lumen areas were 1100 ± 200 and 1300 ± 180 μm^2, respectively ($p < 0.05$ vs. PTCA only). Besides, the data showed whether radiation was used before or after angioplasty, 14 Gy and 20 Gy of radiation could increase the lumen area and decrease the neointimal formation in the model of porcine coronary artery.

The second experiment was the expression of adhesion molecules estimated by RT-PCR at 1 day after angioplasty and/or 20 Gy β-irradiation of pig. The expression of ICAM-1 significantly increased in PTCA only, PTCA/Re (20 Gy), and Re (20 Gy)/PTCA groups rather than in the Re-188 (20 Gy) ($p < 0.001$). The expression of VCAM-1 significantly

decreased in PTCA, Re-188 (20 Gy), and Re (20 Gy)/PTCA groups ($p < 0.01$). The expression of VCAM-1 significantly decreased in PTCA/Re (20 Gy) group ($p < 0.001$).

The third experiment was 1 day after treatment and large-scale gene expression were detected by cDNA microarray. All of the RNA extraction of angioplasty and/or brachytherapy (20) vessels were used. In this study, we screened 440, 246 429, 234 upregulated genes in PTCA, Re-188 (20), PTCA/Re, and Re (20)/PTCA group, respectively. We screened 918, 612, 707, and 653 downregulated genes in the order of groups above. Moreover, according to upregulated and downregulated genes screened, we will identify and confirm through the northern blot or RT-PCR so that we will be able to understand functions and mechanisms of related genes involved in the angioplasty and brachytherapy.

The four experiment was which of VSMCs were received by 0 Gy 2 Gy, and 20 Gy γ-radiation in vitro. The expression of NF-κB, an inducible transcription factor, was estimated by RT-PCR at 1, 2, and 3 day post-radiation. The expression of NF-κB was lower 4 folds in 2 Gy radiated-cells than that in nonirradiated cells at 1 day following radiation. However, the expression of NF-κB increased at 2, and 3 day after radiation. Besides, the results did not show the difference between nonirradiated and radiated VSMCs in the expression of NF-κB at 1, 2, and 3 day after 20 Gy of γ-radiation

Conclusion: We provided a preliminary profile of gene expression for the further analysis. The interests of genes related to the balloon angioplasty and brachytherapy analyzed by cDNA microarray must be confirmed by northern blot and RT-PCR. According to this profile, preliminary results of gene exrpression in the array by which the possible molecular mechanisms in the progression of restenosis and brachytherapy can be investigated.

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Content

Content of Figures

Content of Tables

Abbreviation

Activating transcription factor	ATF
Allograft inflammatory factor-1	AIF-1
Angiotensin converting enzyme	ACE
Apolipoprotein J	Apo J
CAAT/enhancer-binding protein	C/EBP
Cardiovascular disease	CVD
Casein kinase II	CKII
Cis-inducible element	SIE
c-Jun amino terminal kinase	JNK
Coronary artery bypass graft	CABG
CREB-binding protein	CBP
Dithiothreitol	DTT
Double-strand break	DSB
Early growth response factor 1	EGR-1
Endothelial cell growth factor supplement	ECGS
Endothelium-derived-relaxing-factor	EDRF
Ethylenediaminetetraacetic acid	EDTA
Ethyleneglycoltetraacetic acid	EGTA
External elastic lamina	EEL
Extracellular matrix	ECM
Extracellular-signal-regulated kinase	ERK
Fetal bovine serum	FBS
Fibroblast growth factor	FGF
Glycine-rich region	GRR
Glycoprotein Ibα	GP Ibα
Glycoprotein IIb/IIIa	GP IIb/IIIa
Ionizing radiation	IR

N-2-Hydroxyethyl-piperazine-N-2-ethanosulphonic acid	Hepes buffer
IκB kinase	IKK
Immunoglobulin	Ig
IFN-stimulated response element	ISRE
Inducible NO synthase	iNOS
Inhibitory factor kappa B	IκB
Insulin-like growth factor	IGF
Intercellular adhesion molecule	ICAM
Interferon regulatory factor-1	IRF-1
Interleukin-1	IL-1
Internal elastic lamina	IEL
Intravascular bracheytherapy	IVBT
Intravascular ultrasound	IVUS
Lipopolysaccharide	LPS
Lymphocyte function-associated antigen-1	LFA-1
Macrophage-1	MAC-1
Matrix metalloproteinase	MMP
Mitogen activated protein kinase	MAPK
Monocyte specific enhancer binding factor 2c	MEF2C
Myocardial infarction	MI
N-acetyl-L-cysteine or pyrrolidine dithiocarbamate	PDTC
NF-κB-inducible kinase	NIK
Nitric oxide	NO
NO synthase	NOS
Nuclear localization signal	NLS
Oligodeoxynucleotides	ODN
Percutaneous transluminal coronary angioplasty	PTCA

Phenylmethanesulfonyl fluoride	PMSF
Phospatidylinositol-3-kinase	PI3K
Platelet-derived growth factor	PDGF
Platelet-derived growth factor alpha-chain homodimer	PDGF-AA
platelet-derived growth factor beta-chain homodimer	PDGF-BB
Protein kinase A	PKA
Rel homology domain	RHD
Ring-box 1	RBX-1
Serum response element	SRE
Signal transducer and activator transcription	STAT
Single-strand break	SSB
Skp1-cullin 1-f-box protein complex	SCF
Sodium dodecyl sulfate	SDS
12-O-tetradecanoyl-phorbol-13-acetate response element	TRE
Ternary complex factor	TCF
Transforming acidic coiled coil-containing	TACC
Tumor necrosis factor	TNF
(N-Tris[hydroxymethyl]methyl-2-aminoethanesulfonic Acid)	TES
TNF-associated receptor death domain	TRADD
TNF-receptor-associated factor 2	TRAF 2
β-Transducin repeat-containing protein	β-TrCP
Transforming growth factor-β	TGF-β
Tristetraproline	TTP
Vascular cell adhesion molecule	VCAM
Vascular smooth muscle cell	VSMC
Very late antigen-4	VLA-4