

Angiogenic effect of a novel Danshensu
derivative in Zebrafish

By

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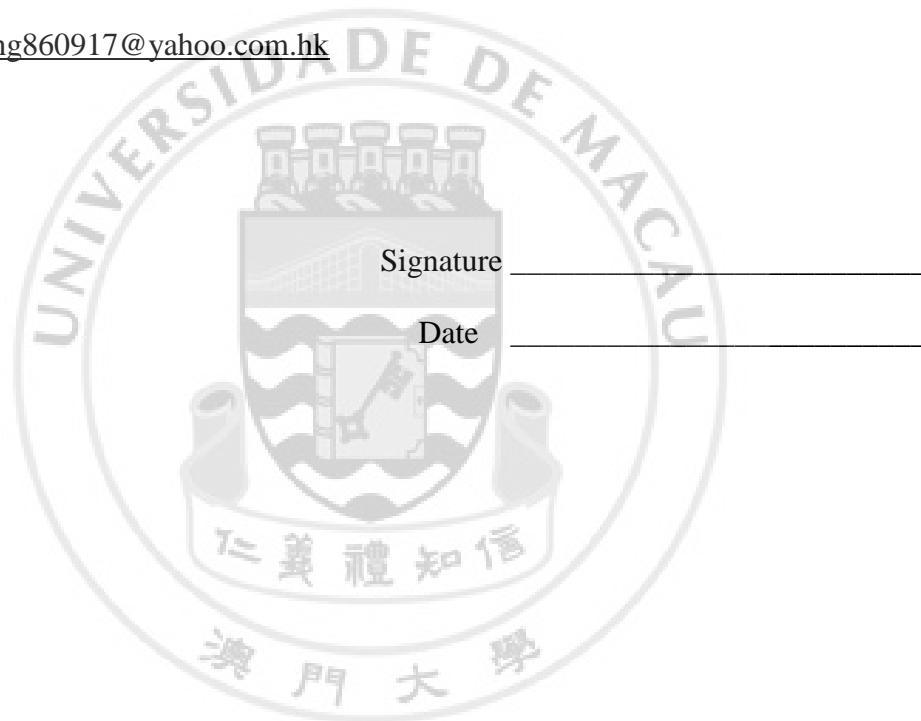
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澳門大學碩士學位論文

新丹參素類衍生物在斑馬魚模型上
促血管新生作用

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中華醫藥研究院

澳門大學

Abstract

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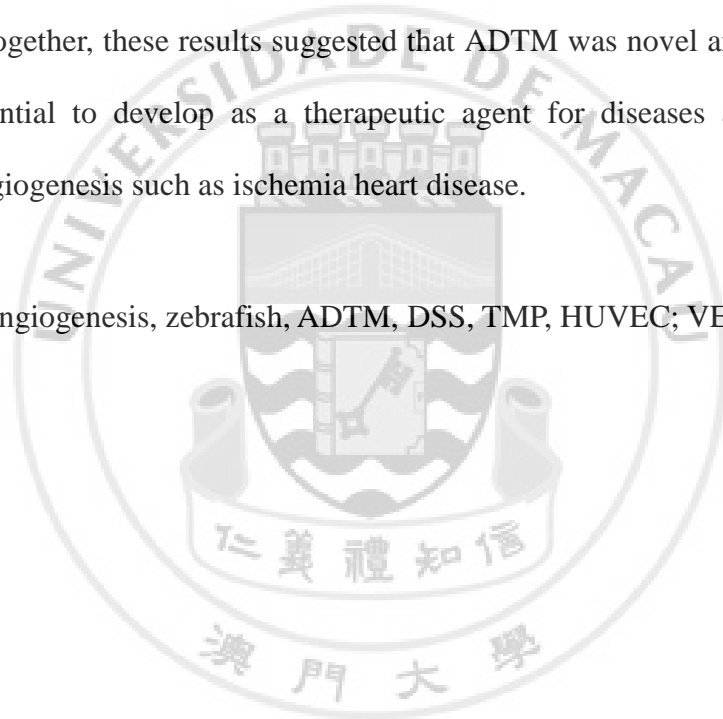
Chinese Medicinal Science

Abstract

Angiogenesis was the formation of new capillary blood vessels from pre-existing vessel, which was an important biological process in physiological and pathological conditions. A variety of diseases such as cancer, diabetic retinopathy, rheumatoid arthritis, ischemic heart disease, chronic wound were associated with dysregulation of angiogenesis. ADTM was new danshensu derivative, synthesized by chemical modification of Danshensu (DSS) and tetramethylpyrazine (TMP), which were active ingredients of Traditional Chinese medicine *Salvia miltiorrhiza* and *Ligusticum wallichii*, respectively. These two herbs were widely used for the treatment of ischemic heart and cerebral vascular disease. In present study, we found that ADTM significantly exhibited pro-angiogenic activity in zebrafish, whereas the underlying mechanism of angiogenesis effect had not been addressed. We investigated angiogenic effect of ADTM using *in vivo* and *in vitro*. The *in vivo* angiogenic effect of ADTM was studied using transgenic *Tg(fli-1a:EGFP)y1* zebrafish embryos by observing the presence of ectopic vessels in the subintestinal vessels (SIV) and recuse

effect of chemical-induced blood vessel loss in intersegmental vessels (ISV) and dorsal longitudinal anastomotic vessel (DLAV) under fluorescence microscopy. ADTM did not exhibit angiogenesis activity in endothelial cell proliferation and capillary tube formation assays, but it significantly reversed VRI-induced blood vessel loss in zebrafish. Furthermore, ADTM also could reverse VRI-induced down-regulation of mRNA expression of VEGFR1 and VEGFR2. This present study proposed the advantage of zebrafish over endothelial cell for screening angiogenic compounds because some compounds were only active in *in vivo* but inactive in *in vitro*. Taken together, these results suggested that ADTM was novel angiogenic agent and had potential to develop as a therapeutic agent for diseases associated with imbalance angiogenesis such as ischemia heart disease.

Key words: angiogenesis, zebrafish, ADTM, DSS, TMP, HUVEC; VEGF.



摘要

血管新生在很多生理過程中扮演著重要的角色，並且與很多疾病有關，例如癌症、糖尿病視網膜病變、類風濕性關節炎、缺血性心臟病、慢性傷口。ADTM，一種新的丹參素衍生物，主要由丹參素和川芎嗪為基本化學結構，經過化學修飾後合成的衍生物。丹參素和川芎嗪分別為傳統中藥丹參和川芎的主要活性成份之一，這兩種中藥在中國數千年以來用作治療缺血性心腦血管疾病。在本次研究中，使用轉基因 *Tg(fli-1a:EGFP)y1* 斑馬魚研究 ADTM 促血管新生作用和其分子機制。

實驗結果發現，ADTM 在斑馬魚模型上具有很強的促血管新生作用。ADTM 能夠逆轉血管內皮細胞生長因子受體抑制劑 (VEGF receptor tyrosine kinase inhibitor, VRI) 誘導體節間血管 (ISV) 和 背部縱向血管 (DLAV) 缺失，且呈劑量依賴關係。同時通過基因表達實驗結果發現，ADTM 能夠逆轉 VRI 引起的 VEGFR1 和 VEGFR2 表達下調。然而，在血管內皮細胞體外模型上沒有顯著的促血管新生作用。本研究提示，ADTM 在斑馬魚體內模型上具有很強的促血管新生作用。另一方面，該研究表明，使用斑馬魚模型篩選血管新生的化合物優越於血管內外細胞模型，且會減少假陰性結果。

關鍵字: 血管新生，斑馬魚，ADTM，丹參素，川芎嗪，血管內皮細胞，血管內皮細胞生長因子

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List of Abbreviations

%	Percent
°C	Degree Celsius
BMD EPCs	Bone marrow-derived endothelial progenitor cells
BrdU	bromodeoxyuridine
CABG	Coronary artery bypass-graft surgery
CHD	Coronary heart disease
DA	Dorsal aorta
DLAV	Dorsal longitudinal anastomotic vessel
DLL4	Delta-like-4 ligand
DMSO	Dimethyl sulfoxide
dpf	Days post fertilization
DSS	Danshensu
EC	Endothelial cell
ECM	Extracellular matrix
EGF	Epidermal growth factor
EGFL7	Epidermal growth factor ligand-7
EGFP	Enhanced green fluorescent protein
FGF	Fibroblast growth factor
FGFR-1	Fibroblast growth factor receptor 1
FRS-2	Fibroblast growth factor receptor substrate 2
Grb14	Growth factor receptor-bound 14
Grb2	Growth factor receptor-bound 2
HER1	Human epidermal growth factor receptor 1
HGF	Hepatocyte growth factor
HP-2	SH2 phosphatase 2
HSPG	Heparan sulfate proteoglycan
HUVEC	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule-1
IHD	Ischemic heart disease
IMG	Intussusceptive microvascular growth
ISV	Intersegmental vessels
KDR	Kinase domain region
min	Minute
ml	Milliliter
mM	Millimole

MAPK	Mitogen activated protein kinase
MEK	MAPK kinase
MMP	Matrix metalloproteinase
MTT	3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltrtrazolium bromide
nM	Nanomole
NO	Nitric oxide
p70 S6K	P70 ribosomal S6 kinase
PA	Plasminogen activator
PAIs	Plasminogen activators inhibitors
PBS	Phosphate-buffered saline
PC	Pericyte
PCA	Percutaneous coronary angioplasty
PCV	Posterior cardinal vein
PDGFB	Platelet-derived growth factor B
PI3k	Phosphoinositide 3-kinase
PIGF	Placenta growth factor
PKC	Protein kinase C
PLA2	Phospholipase A2
PLCg	Phospholipase cg
PLC- γ	Phospholipase C- γ
PLD	Phospholipase D
PM	Plasma membrane
PS	Penicillin-streptomycin
PT-PCR	Reverse transcription polymerase chain reaction
RoBo4	Roundabout homologue-4
RTKs	Receptors tyrosine kinases
Shc	Src homology and collagen
SHP-2	SH2 phosphatase 2
SIV	Subintestinal veins
SPECT	Single photon emission computed tomography
TCM	Traditional Chinese medicine
TIMPs	Tissue inhibitors of mmmps
TMP	Tetramethylpyrazine
t-PA	Tissue-type PA
TSAd	T cell specific adapter
UEA-I	Ulex europas agglutinin I
u-PA	Urokinase-type PA
u-PAR	U-PA binds a cellular receptor

VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VPF	Vascular permeability factors
VSMC	Vascular smooth muscle cell
β -ME	β -mercaptoethano
μ g	Microgram
μ M	Micromole

