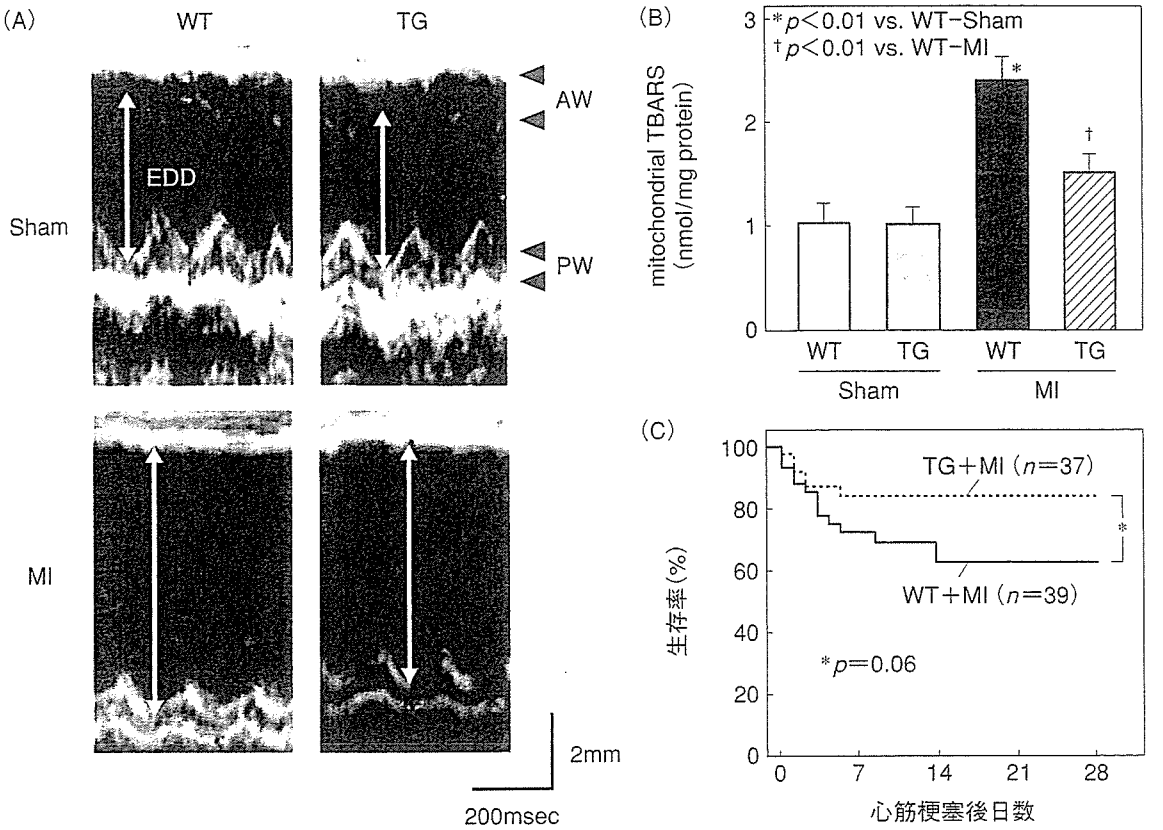


図 2　Prx-3過剰発現マウス

A：心エコー．心筋梗塞(MI)に伴う左室の拡大が抑制された．

B，C：過剰発現マウス(TG)では，野生株(WT)と比べてミトコンドリアでの脂質過酸化の増加が抑制された．

Tfam過剰発現による心不全抑制効果

しかし，さらに効率よくミトコンドリア局所での酸化ストレス産生を抑制できれば，さらに優れた予後改善効果も期待できる．そこで著者らは，ミトコンドリア転写因子A(transcription factor A：Tfam)に注目した．Tfamはmtdnaのtranscription factorとしてFisherらによって最初にクローニングされた[10]．当時，Tfamはミトコンドリア当り15分子程度しか存在しないと信じられてきたが，実はその約100倍量存在すること，その大部分がmtDNAと安定的に結合していることがその後の研究からわかってきた．Tfamはmitochondrial transcription factor B(TFB1M，TFB2M)およびmitochondrial RNA polymerase(POLRMT)とともに，mtDNAの転写に働くことが知られている．

Larssonらが$Tfam^{loxP}$を使い，Tfamノックアウトマウスをそれぞれ作製したところ[11]，$Tfam^{-/-}$ではmtDNAの完全な消失が生じ，胎生8.5～10.5日で死亡し，それらのミトコンドリアは，サイズの拡大，異常クリスタを有し，サブユニットのすべてが核でコードされたsuccinate dehydrogenase(SDH)活性は保たれていたが，mtDNAでコードされたcytochrome *c* oxidase(COX)活性は消失していたことから，Tfamは心不全の形成・進展過程において重要な役割をもつことが示唆された．

そこで，βアクチンをプロモーターにヒトTfamを過剰発現させたマウスを作製した(図3)．本マウスではヒトTfamの発現とともに約40%のmtDNAの増加を認めるが，このTfam過剰発現マウスに心筋梗塞を作製し心筋リモデリングを観察すると，心筋梗塞後もmtDNAコピー数およびミトコンドリア電子伝達系酵素活性が維持され，心筋リモデリングを改善，さらにはその生存率を著しく増加させる(WT 66%，Tfam-TG 100%)ことが明らかとなった(図4)[12]．つまり心筋リモデリングの過程でmtDNAの低下，ミトコンドリア機能の低下が関与しているのに対し，Tfamはmtdnaを維持し，心筋リモデリングを抑制するうえで重要な働きをもつといえる．

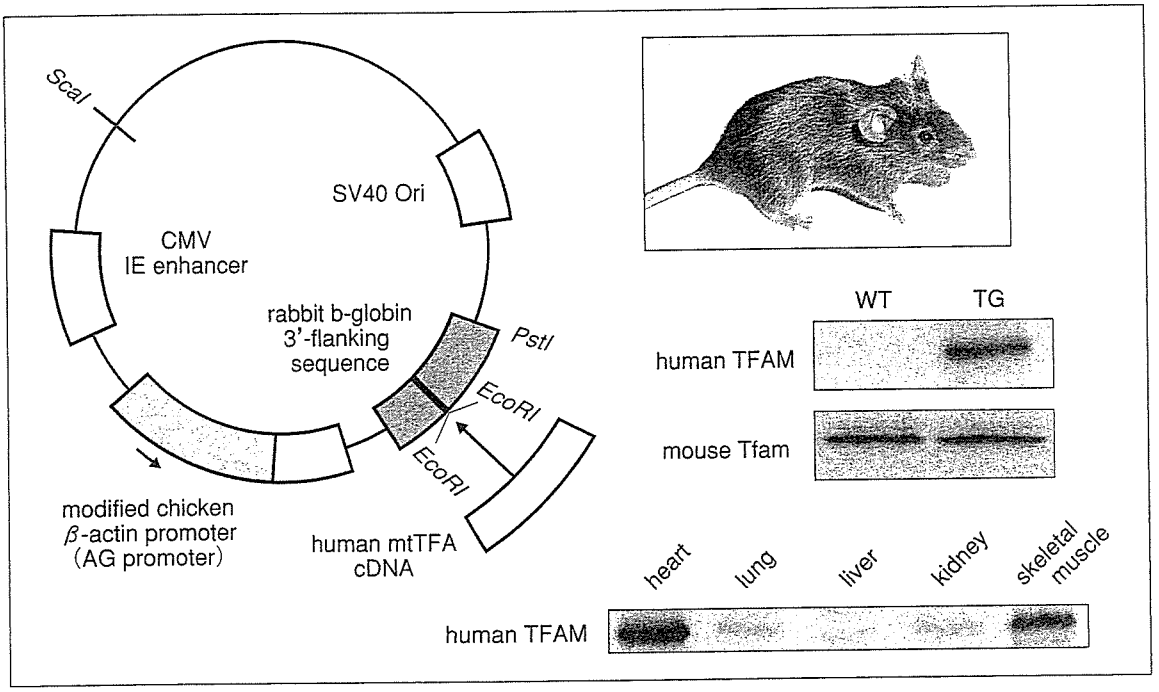


図 3　Tfam過剰発現マウス

ヒト Tfam 過剰発現により内因性 Tfam には影響を及ぼさず，おもに心，骨格筋に強く発現を認めた．

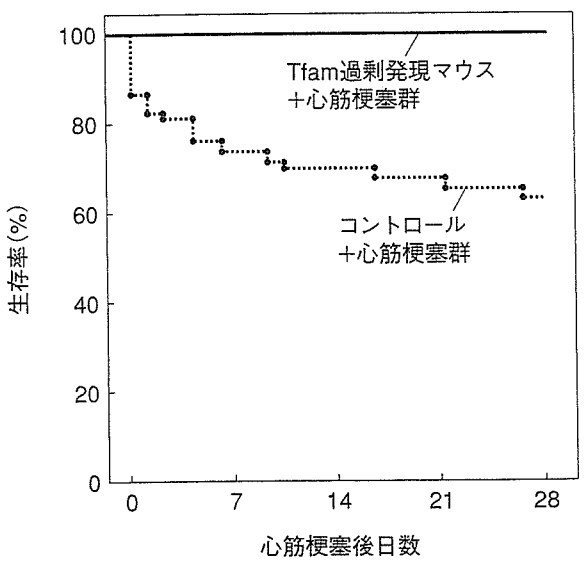


図 4　Tfam過剰発現による心筋梗塞後死亡率の抑制(文献[14]より改変)

Tfam の過剰発現マウスでは mtDNA が増加し，心筋梗塞後の予後を改善した．

おわりに

心不全のあらたな治療ターゲットとして，ミトコンドリア局所での酸化ストレス産生抑制によるリモデリング改善効果について概説した．とくに Tfam はミトコンドリア DNA の維持にきわめて重要で，今後あらたな治療ターゲットとなりうるであろう．Tfam は核でコードされており，その発現は PGC-1，NRF によって制御されている．Tfam を特異的に発現するあらたな手法が今後の臨床応用への発展の鍵となる．

文献

1) Ide, T. et al. : Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. *Circ. Res.*, **86** : 152-157, 2000.
2) Dhalla, A. K. et al. : Role of oxidative stress in transition of hypertrophy to heart failure. *J. Am. Coll. Cardiol.*, **28** : 506-514, 1996.
3) Mallat, Z. et al. : Elevated levels of 8-iso-prostaglandin F2alpha in pericardial fluid of patients with heart failure : a potential role for *in vivo* oxidant stress in ventricular dilatation and progression to heart failure. *Circulation*, **97** : 1536-1539, 1998.
4) Shiomi, T. et al. : Overexpression of glutathione peroxidase prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation*, **109** : 544-549, 2004.
5) Kinugawa, S. et al. : Treatment with dimethylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice : role of oxidative stress. *Circ. Res.*, **87** : 392-398, 2000.
6) Ide, T. et al. : Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. *Circ. Res.*, **85** : 357-363, 1999.
7) Suematsu, N. et al. : Oxidative stress mediates tumor necrosis factor-α-induced mitochondrial DNA damage and dysfunction in cardiac myocytes. *Circulation*, **107** : 1418-1423, 2003.
8) Graham, B. H. et al. : A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a

deficiency in the heart/muscle isoform of the adenine nucleotide translocator. *Nat. Genet.*, **16**：226-234, 1997.
9) Matsushima, S. et al.：Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation*, **113**：1779-1786, 2006.
10) Fisher, R. P. and Clayton, D. A.：Purification and characterization of human mitochondrial transcription factor 1. *Mol. Cell. Biol.*, **8**：3496-3509, 1988.
11) Larsson, N. G. et al.：Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat. Genet.*, **18**：231-236, 1998.
12) Ikeuchi, M. et al.：Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. *Circulation*, **112**：683-690, 2005.

*　　*　　*

特集　慢性心不全の分子機構：新しい治療戦略を求めて

ミトコンドリア傷害と心不全*
—ミトコンドリア由来のレドックス制御による新たな心不全の治療—

井手友美**
筒井裕之***

Key Words : mitochondria, remodeling, oxidative stress, transcription factor A, peroxy-redoxin-3

生活習慣病の増加，および高齢者の比率の上昇に伴って，近年心血管病の発症が増加している．そして，近年の急性期治療の発達と治療効果により，心不全の罹患率は増加の一途をたどり，慢性心不全のメカニズムの解明および治療法の開発が必要不可欠とされてきている．

心不全の病態形成にはレニン-アンジオテンシン，アルドステロンなどの液性因子が関与し，それらが増悪因子となって心肥大・心筋リモデリングが進行し，最終的には非代償的に心筋の破綻が生じることが明らかにされている．さらに，不全心筋において活性酸素(reactive oxygen speicies：ROS)が増加していることが示唆され，心不全の病態形成に酸化ストレスがきわめて重要な役割を果たしていることが示されてきた(図1)．

本稿では，心不全における酸化ストレスの産生メカニズムとその治療法について，最近の知見を概説する．

酸化ストレスの産生源とリモデリングにおける酸化ストレスの関与

ROSとは，通常，スーパーオキサイドアニオン，過酸化水素(H_2O_2)，ヒドロキシラジカル，一重項酸素を意味するが，実際に生体内では，一酸化窒素(NO)や脂質ラジカルなど，多くの酸化ストレスをもたらすものが産生され，それらが相互に作用を及ぼして酸化ストレスを生じていると考えられている．不全心筋で酸化ストレスが増大していることは，種々の心不全動物モデルおよびヒトの心不全においてすでに示されており，また，酸化ストレス消去剤の投与や抗酸化酵素の過剰発現によって心筋リモデリングが抑制されるという結果からも，心不全においてはROSが重要な役割を果たしていると考えられる．そのような酸化ストレスはどこで産生さ

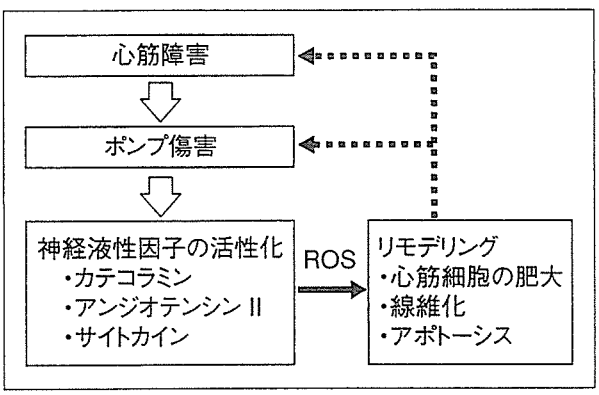


図1　心筋リモデリングの成因
心筋障害から神経液成因子の活性化が生じるが，それにROSが仲介となりリモデリングの増悪，さらなるポンプ傷害へと悪循環が生じる．

* Cardiac remodeling and mitochondrial dysfunction.

** Tomomi IDE, M.D., Ph.D.: 九州大学大学院医学研究院循環器内科学〔〒812-8582 福岡市東区馬出3-1-1〕; Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka 812-8582, JAPAN
*** Hiroyuki TSUTSUI, M.D., Ph.D.: 北海道大学大学院医学研究科循環病態内科学

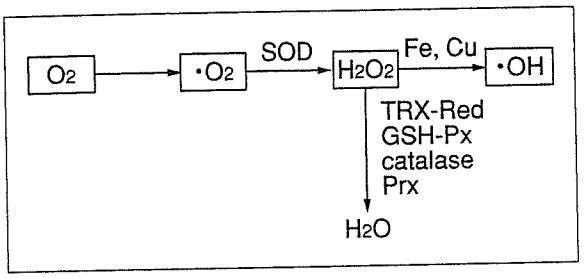


図2 活性酸素産生経路とその代謝
H_2O_2は，ミトコンドリア内ではglutathione peroxidaseやperoxyredoxinなどの酵素反応によって還元される．

れているのであろうか．組織における酸化ストレスの産生源としては，代表的なものとしてNADPHオキシダーゼ，キサンチンオキシダーゼ，およびミトコンドリア電子伝達系がある．なかでも心筋細胞は，そのエネルギー産生を司る細胞内小器官であるミトコンドリアを豊富に有している．ミトコンドリアは細胞のエネルギー代謝を支える「発電所」に相当するところであるが，一方で，そのATP産生のためにミトコンドリアは大量の酸素を代謝しており，それに伴って微量(酸素代謝の1～5％)のスーパーオキサイドが産生され，増加したスーパーオキサイドからミトコンドリア内のMn-superoxide dismutase(SOD)によってH_2O_2に変換され，Harber-Weiss反応または Fenton反応によりハイドロキシラジカルが産生される(図2)．したがって，生体内ではこれらのROSの消去系が必須であるが，その均衡がくずれ，ROSの産生系が有意になった場合，DNA, 脂質，蛋白の傷害が生じると考えられる(図3)．以上のような観点から，酸化ストレスに着目した心不全の新たな治療戦略としては，抗酸化酵素の活性化あるいは，ROS産生の抑制，という主に2つの方法が考えられる．

ミトコンドリア内の抗酸化酵素の増加による心筋リモデリングの抑制

ミトコンドリア内では，Mn-SODやglutathione peroxidase(GPx)に代表されるROS消去酵素群は，心筋リモデリングの抑制に重要な役割を果たしている．心筋梗塞後の心不全マウスではミトコンドリア電子伝達系が傷害され，より多くのROSが産生され脂質過酸化が増加していることから，ミトコンドリア内のROS消去酵素を増加させることで心不全の治療が可能となると考えられる．Peroxyredoxine(Prx)-3はH_2O_2を消去するPrxファミリーの一つで，ミトコンドリアに局在し，酸化ストレスによる神経細胞傷害を抑制することが知られている[1]．このPrx-3を過剰に発現させたマウスに心筋梗塞を作成すると，心筋での酸化ストレスを減少させ，心筋リモデリングを抑制し，心筋梗塞後の予後を改善することが明らかとなった[2](図4)．この結果から，ミトコンドリア内でのROSの消去系を増やすことが，心不全の治療へとつながる可能性があると考えられる．

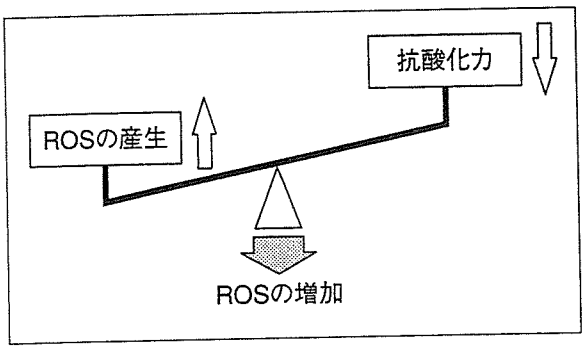


図3 ROSの産生と抗酸化力のバランス
抗酸化力の低下またはROS産生の増加によって，ROSによる組織傷害が生じる．

ミトコンドリア内の活性酸素産生の抑制による心筋リモデリングの抑制

ミトコンドリア内で産生されるROS, つまりスーパーオキサイドを抑制するためには，その電子伝達系の機能を保つことが必要となる．そのためにわれわれは，ミトコンドリア電子伝達系をコードしているミトコンドリアDNA(mtDNA)の傷害を防ぐことが前提になると考えた．mtDNAは，16.5kbの小さな環状二本鎖DNAで，その中にmtDNAの翻訳に必要な22個のtRNA遺伝子，rRNA遺伝子(12S, 16S)，および13個の電子伝達系複合体のサブユニットがコードされている．酸化的リン酸化に必要な呼吸鎖酵素群複合体は，mtDNAと核DNAと両方にコードされたサブユニットから構成されているが，不全心筋ではmtDNAのコピー数が低下し，核のみでコードされたcomplex Ⅱの活性は低下せず，mtDNAと核DNAの両方でコードされたcomplex Ⅰ，Ⅲ，Ⅳの活性が低下しており，ミトコンドリアにおけるROSのターゲットは，mtDNAであることが示唆され

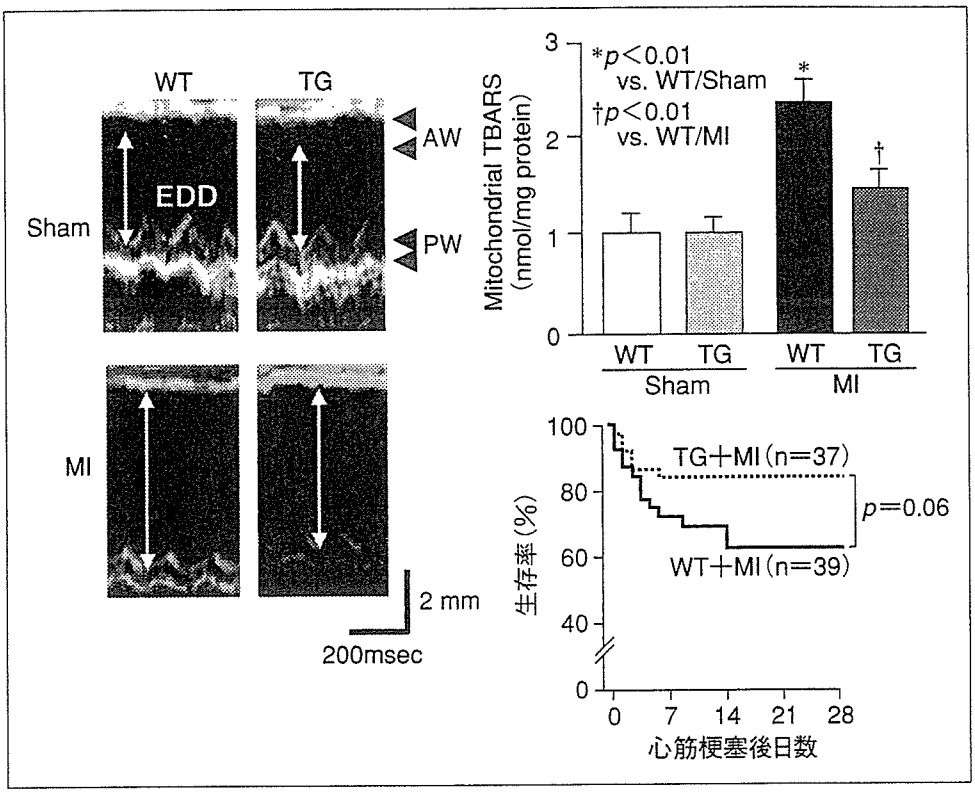


図4　Prx-3過剰発現マウス
心エコーにより，心筋梗塞(MI)に伴う左室の拡大が抑制され，生存率が改善した．過剰発現マウス(TG)では野生株(WT)と比して，ミトコンドリアでの脂質過酸化の増加が抑制された．

る[3]．ミトコンドリア電子伝達系の複合体酵素の活性低下は電子の伝達障害をきたし，さらなるROSの発生をきたす，という悪循環を形成すると考えられ，ROSによる心不全の病態形成の機序の一つであろう(図5)．したがって，この悪循環をたちきることがROSの産生を最小限にする手段であり，そのためにはmtDNAの低下を抑制することが必要となる．また，不全心筋において増加しているサイトカインであるTNFαは，ミトコンドリア呼吸鎖複合体酵素活性を低下させることが明らかで[4]，そのほか，カテコラミン，アンギオテンシンⅡも，心筋細胞からROSを増加させることが示されており，これらのサイトカイン，液性因子も含めて，ミトコンドリア機能障害を含めて，心不全の病態発症・進展へ関与している可能性が示唆されている[5][6]．ミトコンドリア機能については，mtDNAの関与は大きく，主に以下に述べるmtDNAの質的異常と量的異常，つまりコピー数に依存した異常に分けられる．

1．ミトコンドリアDNAの質的な傷害

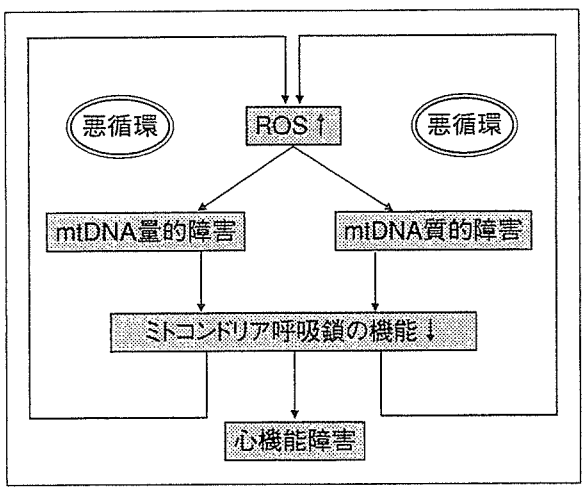


図5　ミトコンドリアDNA傷害と活性酸素の関係
ミトコンドリアDNAの量的・質的な障害によって，ミトコンドリア機能の低下，さらなる活性酸素の産生を生じる．

mtDNAの傷害に伴って心筋症を生じることは，いわゆる突然変異型mtDNAの蓄積によるミトコンドリア病によって知られている．たとえば，特定のtRNA遺伝子のA8322G変異によるものはMERRF(myoclonic epilepsy, myopathy, and

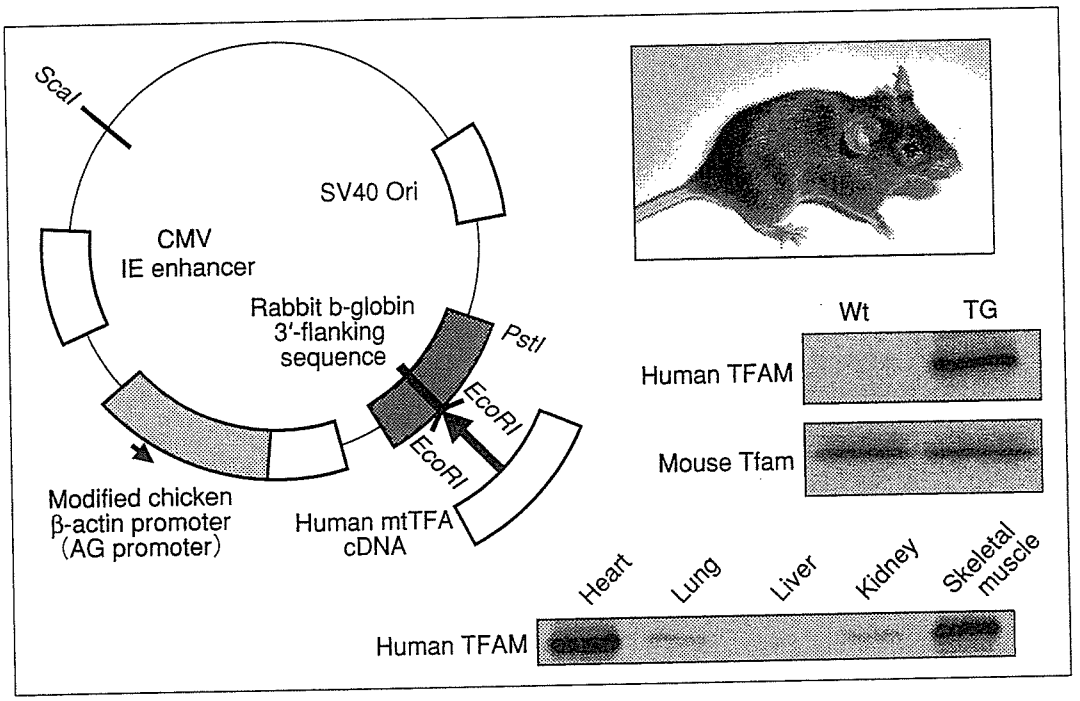


図6　Tfam過剰発現マウス
ヒトTfam過剰発現により，内因性Tfamには影響を及ぼさず，主に，心，骨格筋に強く発現を認めた．

ragged red fiber)と呼ばれ，複合体ⅠおよびⅣの減少を認める．Kearns-Sayre症候群では欠失型突然変異型mtDNAの蓄積が原因となって生じる．これらミトコンドリア遺伝子疾患の発症はmtDNAの突然変異の蓄積によることが知られており，心筋症を含め，脳症や家族性糖尿病など，多彩な病態を示す．現時点では，後天性の心不全に突然変異型mtDNAの蓄積に関する検討は報告されていないが，老化個体や一部の糖尿病でごく少量の突然変異型DNAの検出がなされたことから，ミトコンドリア遺伝子疾患としての心筋症以外にも，mtDNAの突然変異の蓄積が慢性疾患の病態に関与している可能性も示唆されている[7]．

2．ミトコンドリアDNAの量的な傷害

mtDNAは個々のミトコンドリア内に複数存在し，心筋細胞においては1つのミトコンドリアに数百のmtDNAが存在するとされている．不全心筋では，mtDNAコピー数は減少し，さらにmtDNAでコードされている電子伝達系複合体サブユニットのmRNAの低下および複合体酵素活性が低下しているのは，前述のとおりである．しかし，どのようなメカニズムで慢性疾患においてmtDNAのコピー数が減少しはじめるのか，その詳細はいまだ不明な点が多く残されており，今後の課題といえよう．

Mitochondrial transcription factor A (Tfam)の役割

それでは，mtDNAの種々のストレスによる傷害を最小限にするにはどのような手段をとるのがよいのであろうか．

mtDNAの維持およびコピー数の制御に関して，近年，mitochondrial transcription factor A(Tfam)が重要な役割を果たしていることが示唆されている．Tfamは核DNAにコードされた蛋白で，軽鎖および重鎖プロモーターの上流に結合することでmtDNAの転写を制御する[8]．マウスの心筋梗塞後の不全心筋において，mtDNAのコピー数の低下に伴いTfamが低下していることが明らかとなった[9]．そのほか，アドリアマイシン心筋症や糖尿病性心筋症[10]などにおいてもTfamの低下が報告されている．

さらに興味深いことに，心筋特異的または心筋および骨格筋の*Tfam*遺伝子をノックアウトすることにより，拡張型心筋症を生じて心不全をきたすことが報告されている[11][12]．つまりTfamは，mtDNAコピー数の制御およびミトコンドリアの機能維持にきわめて重要な役割を果たし，

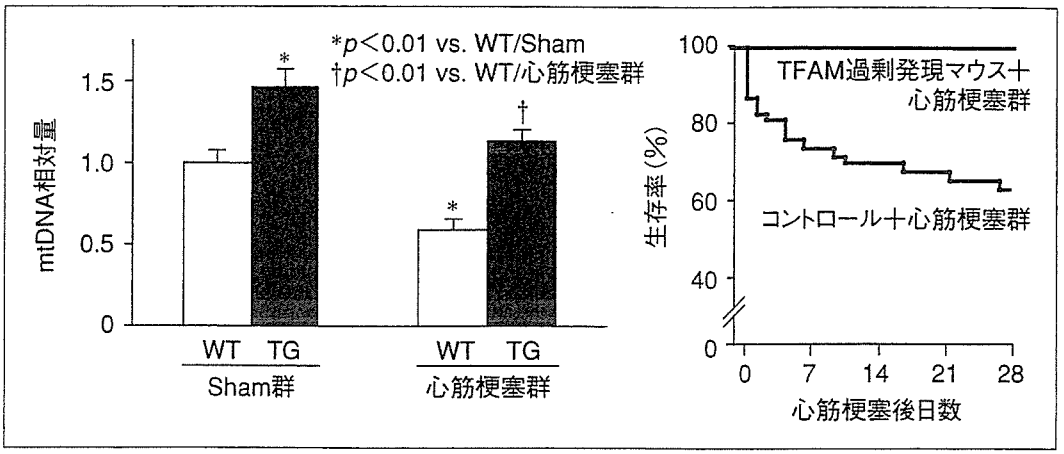


図7 Tfam過剰発現による心筋梗塞後死亡率の抑制
Tfamの過剰発現マウスではmtDNAが増加し，心筋梗塞後の予後を改善した．(文献[14]より改変)

その量的低下はmtDNAの減少を伴い，ミトコンドリア呼吸鎖の機能低下から心機能低下に至ると考えられる．Hela細胞でRNA干渉によってTfamを減少させると，平行してmtDNAも同時に減少し，逆にTfamを過剰発現させるとmtDNAも同時に増加するという現象が認められる[13]．このTfamをβアクチンをプロモーターとして過剰発現させたマウスに心筋梗塞を作成(図6)したところ，mtDNAの増加およびリモデリングの抑制を伴って，著しい予後の改善を認めた(図7)[14]．このTfam過剰発現マウスではmtDNAが約1.5倍増加しており，心筋梗塞作成後もミトコンドリア電子伝達系の機能低下が認められず，Tfamの増加に伴って酸化ストレスの産生が抑制されたことがその機序として考えられる．

このように，酸化ストレスが心筋のリモデリングそして不全心の進展に関与していることから，産生されたROSを制御することが，心不全の治療に有用であることが期待される．とくに，ミトコンドリアを主体とした酸化ストレス産生源の制御が重要であると考えられる．現時点では，Tfamが有効な手段となる可能性は示唆されるものの，それを増加させるための薬剤や方法はみつかっていない．これらのミトコンドリアおよびmtDNAをターゲットとした今後のさらなる研究が必要である．

最後に

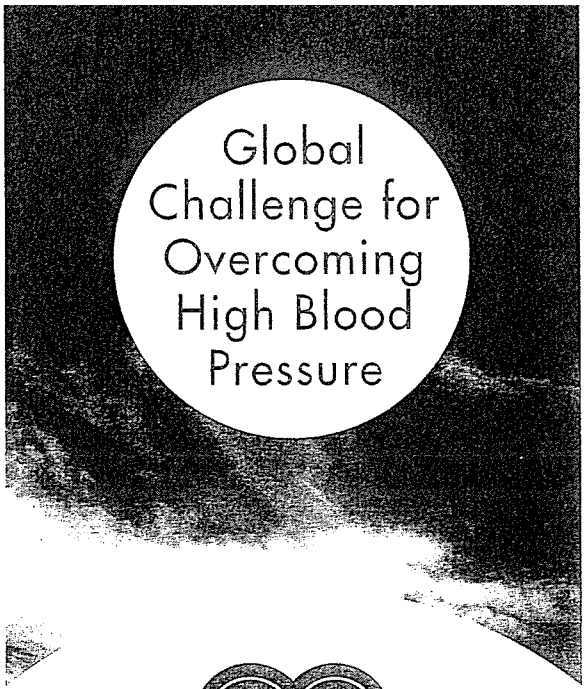
心不全における酸化ストレスの役割について，ミトコンドリアおよびミトコンドリアDNA傷害という観点から最近の知見をまとめた．酸化ストレスは，その作用点，引き金となる産生メカニズムも不明な点が多い．今後さらに酸化ストレスの産生および心筋リモデリング形成へのメカニズムの詳細が明らかとなり，より効率的・効果的なレドックス制御が可能となり心不全の治療へとつながるよう，本分野でのさらなる研究の発展が期待される．

文献

1) Bryk R, Griffin P, Nathan C. Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature 2000；407：211.
2) Matsushima S, Ide T, Yamato M, et al. Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. Circulation 2006；113：1779.
3) Ide T, Tsutsui H, Kinugawa S, et al. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. Circ Res 1999；85：357.
4) Suematsu N, Tsutsui H, Wen J, et al. Oxidative stress mediates tumor necrosis factor-alpha-induced mitochondrial DNA damage and dysfunction in cardiac myocytes. Circulation 2003；107：1418.
5) Sorescu D, Griendling KK. Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure. Congest Heart Fail 2002；8：132.

6) Kakishita M, Nakamura K, Asanuma M, et al. Direct evidence for increased hydroxyl radicals in angiotensinII-induced cardiac hypertrophy through angiotensinII type 1a receptor. J Cardiovasc Pharmacol 2003 ; 42 Suppl 1 : S67.
7) Wallace DC. Mitochondrial diseases in man and mouse. Science 1999 ; 283 : 1482.
8) Fisher RP, Clayton DA. Purification and characterization of human mitochondrial transcription factor 1. Mol Cell Biol 1988 ; 8 : 3496.
9) Ide T, Tsutsui H, Hayashidani S, et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 2001 ; 88 : 529.
10) Kanazawa A, Nishio Y, Kashiwagi A, et al. Reduced activity of mtTFA decreases the transcription in mitochondria isolated from diabetic rat heart. Am J Physiol Endocrinol Metab 2002 ; 282 : E778.
11) Li H, Wang J, Wilhelmsson H, et al. Genetic modification of survival in tissue-specific knockout mice with mitochondrial cardiomyopathy. Proc Natl Acad Sci USA 2000 ; 97 : 3467.
12) Wang J, Wilhelmsson H, Graff C, et al. Dilated cardiomyopathy and atrioventricular conduction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. Nat Genet 1999 ; 21 : 133.
13) Kanki T, Ohgaki K, Gaspari M, et al. Architectural role of mitochondrial transcription factor A in maintenance of human mitochondrial DNA. Mol Cell Biol 2004 ; 24(22) : 9823.
14) Ikeuchi M, Matsusaka H, Kang D, et al. Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. Circulation 2005 ; 112 : 683.

* * *



ISH 2006

October 15 - 19, 2006 Fukuoka, Japan

The 21st Scientific Meeting of the International Society of Hypertension

5th Asian-Pacific Congress of Hypertension

29th Annual Scientific Meeting of the Japanese Society of Hypertension

Abstracts

OS33-3 AMLODIPINE-INDUCED REDUCTION OF OXIDATIVE STRESS IN THE BRAIN IS ASSOCIATED WITH SYMPATHO-INHIBITORY EFFECTS IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

Yoshitaka Hirooka, Yoshikuni Kimura, Masatsugu Nozoe, Yoji Sagara, Koji Ito, Kenji Sunagawa
Kyushu University Graduate School of Medical Sciences, Japan

Objectives: Amlodipine is a dihydropyridine calcium channel blocker that is widely used for the treatment of hypertensive patients and has an antioxidant effect on vessels in vitro. The aim of the present study was to examine whether treatment with amlodipine reduced oxidative stress in the brain of stroke-prone spontaneously hypertensive rats (SHRSP). **Design, Methods, and Results**: The animals received amlodipine, nicardipine, or hydralazine for 30 days in their drinking water. Levels of thiobarbituric acid-reactive substances (TBARS) in the brain (cortex, cerebellum, hypothalamus, and brainstem) were measured before and after each treatment. Systoloic blood pressure decreased to similar levels in the amlodipine-, nicardipine-, and hydralazine-treated groups. Urinary norepinephrine excretion was significantly reduced in SHRSP after treatment with amlodipine, but not in nicardipine or hydralazine. Levels of TBARS in the cortex, cerebellum, hypothalamus, and brainstem were significantly higher in SHRSP than in Wistar-Kyoto rats (WKY), and were reduced in amlodipine-treated, but not in nicardipine- or hydralazine-treated SHRSP. Electron spin resonance spectroscopy revealed increased levels of reactive oxygen species in the brain of SHRSP, which were reduced by treatment with amlodipine. Intracisternal infusion of amlodipine also reduced systolic blood pressure, urinary norepinephrine excretion, and the levels of TBARS in the brain. **Conclusions**: These results suggest that oxidative stress in the brain is enhanced in SHRSP comp WKY. In addition, ahtihypertensive treatment with reduces oxidative stress in all areas of the brain examined and decreases blood pressure without a reflex increase in sympathetic nerve activity in SHRSP.

OS33-4 SYSTEMIC HAEMODYNAMICS AND LEFT VENTRICULAR HYPERTROPHY IN SHR WITH ADRIAMYCIN NEPHROPATHY: SHORT TERM AND LONG TERM EFFECTS OF LOSARTAN TREATMENT

Nevena_D Mihailovic-Stanojevic[2], Djurdjica_M Jovovic[1], Zoran_Z Miloradovic[1], Dragan Mitrovic[2], Jasmina Markovic-Lipkovski[2]
[1]Institute for Medical Research, Yugoslavia, [2]Institute of Pathology, School of Medicine, Belgrade, Serbia and Montenegro
nevena@imi.bg.ac.yu

OBJECTIVE: Patients with chronic renal failure have a high risk for cardiovascular disorders. The aim of our study was to examine effects of adriamycin (ADR) as well as short term and long term effects of losartan on systemic haemodynamics and left ventricular hypertrophy in SHR with ADR nephropathy.METHODS: Female SHRs, 24 weeks old, were selected in 6 groups: Control groups (C6, C12) without treatment. Groups ADR6, ADR-L6 and ADR12, ADR-L12 received ADR (2mg/kg/b.w.i.v.) twice in interval of 3 weeks. Group ADR-L6 received losartan (10mg/kg/b.w./day, by gavages) for 6 and group ADR-L12 for 12 weeks after ADR. Six and 12 weeks after adriamycin treatment animals were anesthetized and systolic, diastolic and mean blood pressure, cardiac index (CI), total peripheral resistance (TPR), and left ventricle mass index (LVMI) were measured.RESULTS: LVMI was significantly increased in ADR6 group compared to C6, (0.44 ± 0.01 vs. 0.35±0.01, $p<0.05$), without changes in systemic haemodynamics. Losartan significantly decreased LVMI independently of treatment period, but long term effects was more pronounce (ADR-L6 vs. ADR6, 0.36 ± 0.01 vs. 0.44 ±0.01, and ADR-L12 vs. ADR12, 0.33 ± 0.01 vs. 0.38 ±0.01, ADR-L12 vs. ADR-L6, $p<0.05$). Blood pressure was reduced after short treatment with losartan. In ADR12 group CI was increased and TPR was decreased compared to C12. Long term losartan treatment returns these parameters to control values.CONCLUSION: Losartan prevents left ventricular hypertrophy independent from systemic haemodynamics and treatment duration in SHR with ADR nephropathy.

OS33-5 (PRO)RENIN RECEPTOR: BIOCHEMICAL STUDY ON THE BINDING TO PRORENIN AND RENIN

A.H.M._Nurun Nabi[1], Mohammad_Nasir Uddin[2], Tsutomu Nakagawa[2], Atsuhiro Ichihara[3], Tadashi Inagami[4], Fumiaki Suzuki[1,2]
[1]The United Graduate School of Agricultural Science, Gifu University, Gifu, Japan, [2]Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan, [3]Keio University School of Medicine, Tokyo, Japan, [4]Biochemistry School of Medicine, Vanderbilt University, Nashville, TN, USA
nurunnabi369@yahoo.com

Objective: The binding and activation mechanism of renin and prorenin by its receptor was investigated to evaluate the role of 'handle' region in prorenin.*Design & Methods*: This study was performed by using membrane anchored rat and human (pro)renin receptors. The plasmid DNA containing human and rat (pro)renin receptors (AF291814 and AB188298 in DDBJ, respectively) conjugated with FLAG epitope were transfected into the COS-7 cells. Synthetic peptides 10P-19P and the 11P-15P were used at 4.0 nM. *Results*: The human and rat (pro)renin receptor was expressed on the membrane. The highest amount of receptor was found after 18h transfection. Human/rat renin and prorenin bound to the receptor with different binding affinities. The 50% of the total human prorenin and 90% of the total rat prorenin at 2.0 nM bound to the receptors (K_d were 1.8 and 0.89 nM, respectively). Receptor-bound rat prorenin was activated non-proteolytically to an enzymatically active form with a K_m of 1.0 mM, and a V_{max} 30% of V_{max} attained by mature renin. That of human prorenin had the renin activity at the similar level to that of mature renin. The peptides inhibited the binding of both human and rat prorenin to the respective membrane-anchored receptors and their Ki were around 7 nM for both peptides. *Conclusion*: (Pro)renin receptor activates human and rat prorenin non-proteolytically and possibly the 'handle' region plays a crucial role by opennig the active site of prorenin in the "receptor associated prorenin system" (RAPS). This receptor also bound renin from human and rat source.

OS33-6 ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKER, CANDESARTAN AMELIORATES COGNITIVE IMPAIRMENT IN TYPE-2 DIABETES MICE WITH IMPROVEMENT OF INSULIN RESISTANCE

Kana Tsukuda, Masaki Mogi, Jian-Mei Li, Jun Iwanami, Li-Juan Min, Rui Chen, Masaru Iwai, Masatsugu Horiuchi
Department of Molecular Cardiovascular Biology and Pharmacology, Ehime University, Graduate School of Medicine, Tohon, Ehime, Japan
mmogi@m.ehime-u.ac.jp

Objective: It has been reported that type-2 diabetes mellitus is closely related to diminished cognition and ARB have been shown to suppress new onset of diabetes mellitus and improve insulin resistance. We examined the changes in age-dependent cognitive function in KK-Aʸ mice, a model of type-2 diabetes, and the potential effects of ARB on amelioration of cognitive function.
Design and Methods: To assess the cognitive function, mice were subjected to a passive avoidance task every week from 8 weeks-old. Expression of MMS2, one of the ubiquitin-conjugating enzyme variants, and AT_2 receptor was analyzed by real-time PCR. Glucose-uptake was evaluated using 2-deoxy-D-[^{3}H] glucose.
Results: In KK-Aʸ mice, ARB, candesartan orally administrated at a non-hypotensive dose significantly decreased serum glucose and insulin levels. Glucose-uptake in brain was increased by candesartan treatment. Avoidance rate was increased age-dependently in non-diabetic C57BL/6J mice, but not in KK-Aʸ mice. Cognitive impairment was observed after 14 weeks-old. Administration of candesartan from 8 weeks-old markedly improved cognitive function and administration from 14 weeks-old prevented further cognitive decline. We demonstrated that AT_2 receptor signaling enhances neural differentiation and prevents cognitive decline after stroke in mice via an increase in MMS2. MMS2 mRNA expression was significantly attenuated in the brain of KK-Aʸ mice compared to that in C57BL/6J mice. Treatment with candesartan increased MMS2 mRNA expression as well as AT_2 receptor expression.
Conclusions: Candesartan ameliorated the impaired cognitive function in type-2 diabetes mice at least due to improvement of insulin resistance and increase in MMS2 expression.

PM3-03-02 ORAL TREATMENT WITH AZELNIDIPINE REDUCED RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) AND IMPROVED NONLINEAR CORRELATION BETWEEN RSNA AND BP IN CONSCIOUS SHR

Toshiko Onami[1], Hiroo Kumagai[1], Chie Takimoto[1], Motohisa Osaka[2], Katsufumi Sakata[1], Kamon Iigaya[1], Koichi Hayashi[1], Hiroshi Itoh[1], Takao Saruta[1]
[1]*Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan,* [2]*Department of Gerontology, Nippon Medical School, Kawasaki, Japan*

Objective: Faster heart rate (HR) and potentiated sympathetic activity are risk factors for CV events. Post myocardial infarction patients with a reduced nonlinearity of HR regulation showed a poor survival rate. Azelnidipine (a novel DHP-CCB) reduced HR in 101 Japanese hypertensive patients. We demonstrated in ISH 2004 that acute intravenous azelnidipine reduced HR and RSNA despite significant depressor effect in SHR. Design and Methods: To examine the effects of azelnidipine on linear and nonlinear correlations between RSNA and BP, we recorded simultaneously BP, HR, RSNA, and RBF in conscious SHR (n=8) before (13 week-old) and after 2-week oral treatment (through gastric tube) with azelnidipine (1.5mg/kg/day dissolved in DMSO). Coherence of transfer function from RSNA to BP was calculated as linearity. Mutual information value (developped by Dr Osaka) between RSNA and BP was calculated as linearity and nonlinearity. Results: Despite significant depressor effect from 160±5 to 133±2 mmHg , HR reduced from 398±7 to 391±5 beats/min, and RSNA decreased from 15±2 to 9.6±0.8 ($p<0.05$). RBF tended to increase. The coherence (linearity) was significantly reduced from 0.7±0.04 to 0.6±0.03 (0-0.5 Hz). Mutual information value tended to increase from 0.2±0.01 to 0.3±0.01, suggesting an increase in nonlinear correlation. Conclusions: Two-week oral treatment with azelnidipine did not induce reflex tachycardia, significantly reduced RSNA, and increased the nonlinear correlation between RSNA and BP. Therefore, azelnidipine has a potential to prevent CV events in hypertensive patients.

PM3-03-03 DO DISTINCT POPULATIONS OF DORSAL ROOT GANGLION NEURONS ACCOUNT FOR THE HIGH SENSITIVITY OF CGRP CONTAINING RENAL AFFERENT NERVE FIBERS

Peter Linz[1], Claudia Heersink[1], Eva Vogel[2], Gisa Tiegs[2], Kerstin Amann[3], Karl_F Hilgers[1], Roland_E Schmieder[1], Roland Veelken[1]
[1]*University of Erlangen; Medical Department 4, Nephrology and Hypertension,* [2]*University of Erlangen; Department of Pharmacology,* [3]*University of Erlangen; Department of Pathology, Germany*
mfm431@rzmail.uni-erlangen.de

The neurogenic peptide CGRP was linked to kidney damage in hypertension. CGRP is locally released from sensory afferent fibers also transducing information to the brain. The receptors involved in the stimulation of sensory neurons in the kidney cortex are not well investigated.Our hypothesis: The stimulation of a distinct population of very sensitive dorsal root ganglion neurons (DRGN) with afferent input from the renal cortex depends on TRPV1 [capsaicine] receptors associated with these fibers.Identified rat DRGN with renal axons were studied by patch-clamp techniques. (DRGN with hind limb afferents studied for comparison). Renal DRGN showed significantly higher amplitudes of acid induced transient and sustained currents than non-renal DRGN; transient: (15.99±5.05 pA/pF vs 0.36 ±0.17* pA/pF, mean±SEM at pH 6) sustained: (3.67±1.13 pA/pF vs 0.64±0.14* pA/pF at pH 6 and 20.04±4.51 pA/pF vs 6.22±1.18* pA/pF at pH 5) (* $p<0.05$, n = 25). The TRPV1 receptor antagonist capsazepine inhibited the sustained, amiloride the transient responses suggesting involvement of Acid Sensing Ion Channels (ASIC). Furthermore, small-capacitance renal DRGN ($p<80$pF) showed no ASIC- or TRPV1-currents wheras larger-capacitance renal DRGN (>80pF) exhibited strong currents which were significantly higher than in non-renal DRGN. Confocal microscopy revealed that renal vessels, tubuli and glomeruli were associated with CGRP-containing nerves possessing TRPV1 and ASIC receptors. Sensory afferent neurons innervate the kidney cortex. Distinct large-capacitance neurons exhibit high sensitivity to stimulation with protons. These neurons may play a pivotal role for the transduction of afferent input to brain, and for the local release of peptides such as CGRP.

PM3-03-04 ESTROGEN DEPLETION INDUCED HYPERTENSION THROGH RHO-KINASE ACTIVATION IN THE BRAAINSTEM IN FEMALE SPONTANEOUSLY HYPERTENSIVE RATS

Koji Ito[1], Yoshitaka Hirooka[2], Akira Takeshita[2], Kenji Sunagawa[2]
[1]*kyushu kousei-nenkin hospital / Japan,* [2]*Departement of cardiovascular medicine, Kyushu university, Japan*

Estrogen decreases arterial pressure by acting directly on blood vessels and through the effects on cardiovascular centers in the brainstem. The mechanism underlying the effects of estrogen in the brainstem, however, is not clear. The aim of the present study was to determine whether Rho-kinase in the brainstem contribute to ovariectomy-induced hypertension. We performed bilateral ovariectomy or sham operation in 12-week-old female spontaneously hypertensive rats (SHR). Arterial pressure and heart rate (HR) measured using radio-telemetry in conscious state, were increased in ovariectomized-rats compared with control-rats. Continuous intracisternal infusion of Y-27632, a specific Rho-kinase inhibitor, via a mini-osmotic pump significantly attenuated the ovariectomy-induced increase in arterial pressure and HR. In addition, we compared the Rho-kinase activity in the brainstem between control-rats and ovariectomized-rats. The levels of phosphorylated-ERM family (ezrin, radixin, and moesin), Rho-kinase target proteins were significantly greater in ovariectomized-rats than in control-rats. Intracisternal infusion of Y-27632 attenuated the expression of phosphorylated-ERM family. Furthermore, we examined the estrogen (E2) concentration in serum and CSF. The ovariectomy decreased E2 concentration both in serum and CSF to about 25% of control. These results suggest that the depletion of endogenous estrogen by ovariectomy, at least in part, induces hypertension in female SHR via activation of the Rho-kinase pathway in the brainstem.

PM3-03-05 INCREASED REACTIVE OXYGEN SPECIES IN NUCLEUS TRACTUS SOLITARII IS INVOLVED IN NEURAL MECHANISMS OF HYPERTENSION IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

Masatsugu Nozoe, Yoshitaka Hirooka, Yasuaki Koga, Yoji Sagara, Takuya Kishi, Kenji Sunagawa
Kyushu University Graduate School of Medical Sciences, Japan

Objective: We reported that increased reactive oxygen species (ROS) in the rostral ventrolateral medulla (RVLM) contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats (SHRSP). However, the role of ROS in the nucleus tractus solitarii (NTS), where afferent input from baroreceptors receives, in cardiovascular regulation is not understood. The aim of the present study was to examine whether ROS generation in the NTS is involved in neural mechanisms of hypertension. Design and Methods: ROS generation in the NTS of SHRSP and WKY was evaluated measurement of thiobarbituric acid-reactive substances (TBARS) levels. The role of ROS in the NTS of SHRSP in cardiovascular regulation was evaluated by two different methods; transfection of either adenovirus encoding human Cu/Zn Superoxide dismutase (AdCu/ZnSOD) to scavenge superoxide of cytozole or adenovirus encoding dominant-negative Rac1 (AdDN17Rac1) to suppress ROS generation by NAD(P)H oxidase into the NTS. Mean blood pressure (MBP) and heart rate (HR) were measured using radio-telemetry system. Urinary norepinephrine excretion for 24 hours was measured as an indicator of sympathetic nerve activity.Results: TBARS levels and NAD(P)H oxidase activity in the NTS were greater in SHRSP than in WKY. Cu/ZnSOD activity was lower in SHRSP than in WKY. Transfection of either AdCu/ZnSOD or AdN17Rac1 into the NTS decreased MBP, HR, and urinary norepinephrine excretion in SHRSP.Conclusions: These results suggest that the increased ROS generation probably via NAD(P)H oxidase in the NTS is increased in SHRSP, and contribute, at least in part, to neural mechanisms of hypertension.

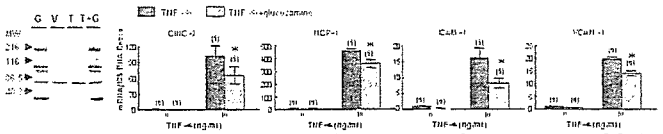


*Figure Western blot (probed with selective primary CTD-110.6 antibody) Glucosamine enhances O-GlcNAc protein levels in RASMCs G, glucosamine, T, TNF-α, Bar graph Glucosamine (5 mM, 1 hr pretreatment) on mRNA expression of chemokines [cytokine-induced neutrophil chemoattractant (CINC)-2β and monocyte chemotactic protein (MCP)-1] and adhesion molecules [intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VACM)-1] in RASMCs stimulated with TNF-α (10 ng/ml, for additional 6 hrs). Data, expressed as means±SEM, are from real time quantitative RT-PCR assay are standardize to the mean mRNA level of the V-treated RASMCs. (n)=number of wells. *p<0.05 vs respective V-treated RASMCs, # p<0.05 vs respective TNF-α-treated RASMCs.*

PM4-01-08 ENHANCED ERYTHROPOIESIS MEDIATED BY ACTIVATION OF THE RENIN-ANGIOTENSIN SYSTEM VIA ANGIOTENSIN II TYPE 1A RECEPTOR

Hideki Kato[1], Junji Ishida[2], Takeshi Sugaya[2], Keiji Tanimoto[2], Takashi Yokoo[3], Toshiro Fujita[1], Masaomi Nangaku[1], Akiyoshi Fukamizu[2]
[1]Division of Nephrology and Endocrinology, Tokyo University Hospital, Tokyo, Japan, [2]Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Japan, [3]Division of Nephrology and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan

GFG07102@nifty.com

Objective: Although clinical and experimental studies have long suggested a role for the renin-angiotensin system (RAS) in the regulation of erythropoiesis, the molecular basis of this role has not been well understood.
Design and Methods: We report here that transgenic mice carrying both the human renin and human angiotensinogen genes displayed persistent erythrocytosis as well as hypertension. To identify the receptor molecule responsible for this phenotype, we introduced both transgenes into the AT1a receptor-null background. Angiotensin II has been shown to influence erythropoiesis by two means, up-regulation of erythropoietin levels and direct stimulation of erythroid progenitor cells. Thus, we conducted bone marrow transplantation experiments. Plasma erythropoietin levels and kidney erythropoietin mRNA expression were measured by ELISA and quantitative PCR, respectively.
Results: We found that elevated hematocrit level was restored to the normal level in the double transgenic mice in the AT1a receptor-null background by genetic manipulations and clarified that AT1a receptors on bone marrow-derived cells were dispensable for RAS-dependent erythrocytosis by bone marrow transplantation experiments. Plasma erythropoietin levels and kidney erythropoietin mRNA expression in the double transgenic mice were significantly increased compared to those of the wild-type control, while the elevated plasma erythropoietin levels were significantly attenuated in the compound mice.
Conclusions: RAS enhances erythropoiesis through the AT1a receptor of kidney cells and that this effect is mediated by the elevation of plasma erythropoietin levels in vivo.

PM4-01-09 CALCITRIOL, AND THE LESS CALCEMIC ANALOG QW-1624F$_2$-2, LOWER BLOOD PRESSURE IN THE TSUKUBA HYPERTENSIVE MOUSE, A TRANSGENIC MODEL OF HYPERTENSION DUE TO EXPRESSION OF THE HUMAN RENIN-ANGIOTENSIN SYSTEM.

Karen M. Tordjman[1], Jessica Sack[1], Dalia Somjen[1], Gary H. Posner[2], Michal Vechoropoulos[1], Naftali Stern[1]
[1]Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel, [2]Department of Chemistry, Johns Hopkins University, Baltimore, Maryland, USA

karentor@tasmc.health.gov.il

Epidemiologic studies have suggested a relationship between vitamin D levels and blood presssure (BP). It was recently demonstrated that calcitriol downregulates the expression of the renin gene.
In the present study we tested the hypothesis that calcitriol, and its less calcemic analog QW-1624F$_2$-2, could attenuate the hypertension in the Tsukuba Hypertensive Mouse (THM). Newly weaned, 4 week-old THM mice received intraperitoneal injections of either calcitriol or QW-1624F$_2$-2(0.5ng/g body weight). Within 3 weeks, systolic BP was 122.8±4.1 mm Hg, in the calcitriol-treated animals, and 113.2±2.8 mm Hg in QW-1624F$_2$-2-treated mice, vs. 150.8±6.8 mm Hg in the control (vehicle-injected) animals (P<0.001, by ANOVA). In a separate experiment, treatment with QW-1624F$_2$-2 was initiated in frankly hypertensive 6 week old animals.
After 3 weeks of treatment, BP was down to 112.6±6.5 mm Hg in the analog-treated mice (P<0.005, by ANOVA vs. baseline), but unchanged in controls. None of the treatments was associated with significant hypercalcemia. Echoing the reduction in BP, expression of the transgene human renin mRNA was down by approximately 65% in the kidneys of the calcitriol and the analog-treated mice compared to control (P=0.03). In addition, the level of the mouse angiotensin II receptor type 1 mRNA was decreased by 35% in the aorta.
As renin is the rate limiting step of the renin-angiotensin system, our data support the notion that vitamin D modulates this system in vivo. If confirmed, these results suggest a potential therapeutic role for calcitriol and its less calcemic analogs in the treatment of hypertension and cardiovascular diseases.

PM4-02-01 ALDOSTERONE SUTIMULATES SUPEROXIDE ANION PRODUCTION IN BOVINE AORTIC ENDOTHELIAL CELLS

Fumiko Iwashima, Takanobu Yoshimoto, Haruna Sakai, Toru Sugiyama, Yukio Hirata
Tokyo Medical and Dental University graduate school, Japan

Objective : It is well recognized that angiotensin II (Ang II) increases vascular NAD(P)H oxidase expression, thereby leading to increased oxidative stress in vascular cells, while aldosterone is currently considered as a cardiovascular risk hormone. The aim of present study was to elucidate whether aldosterone directly stimulates superoxide anion production in bovine aortic endothelial cells (BAEC), and if so, to compare the differential effects by aldosterone and Ang II on superoxide anion production.
Design and Methods : Primary culture of BAEC (passage : 2-8) were used for the experiments. Intracellular generation of superoxide anion in BAEC treated with aldosterone and Ang II was measured by dihydroethydium (DHE) fluorescence using fluorescent microscopy and image analyzing system.
Results : Aldosterone (10^{-8} M) significantly increased superoxide anion levels in BAECs in a time-dependent manner (3-24 hrs); a significant increase was induced as early as 3 hrs, and about 3-4 fold increase by 24 hrs. In contrast, Ang II (10^{-7} M)-stimulated increase in superoxide anion levels was biphasic; the first phase was observed as early as 30 min, and the later sustained phase by 3-24 hrs. The aldosterone-induced generation of superoxide anion was inhibited by mineralocorticoid receptor antagonist, eplerenone, but not by Ang II type 1 receptor antagonist, varsaltan. Superoxide anion generation by aldosterone and Ang II was equally inhibited by NAD(P)H oxidase inhibitor, apocynin ($3*10^{-4}$ M).
Conclusions : Our study clearly demonstrates that aldosterone directly stimulates superoxide anion generation in endothelial cells via NAD(P)H-dependent pathway independent from Ang II effect.

PM4-02-02 HIGH SALT DIET INCREASES BLOOD PRESSURE VIA OXIDATIVE STRESS IN THE BRAINSTEM IN SPONTANEOUSLY HYPERTENSIVE RATS

Yasuaki Koga, Yoshitaka Hirooka, Shuichirou Araki, Masatsugu Nozoe, Yoji Sagara, Takuya Kishi, Kenji Sunagawa
Kyushu University Graduate School of Medical Sciences, Japan

yasukoga@cardiol.med.kyushu-u.ac.jp

Objective: High dietary sodium causes sympathetic hyperactivity and increases resting blood pressure (BP) in spontaneously hypertensive rats (SHR). In part, central nervous system mechanisms are suggested to be involved. Recent studies suggest that reactive oxygen species (ROS) generation in the brainstem is increased in SHR. The aim of the present study was to examine whether high salt diet increases ROS in the rostral ventrolateral medulla (RVLM) of SHR and, if so, this is associated with BP elevation.
Design and Methods: Male SHR (6-week-old) were fed with high salt diet (8%: HS) or low salt diet (0.5%: LS) for 6 weeks. BP was measured using the tail-cuff method. Norepinephrine excretion was measured at 6-week-old and 12-week-old. We evaluated ROS in the brain (cortex, cerebellum, hypothalamus and RVLM) by thiobarbituric acid-reactive substances (TBARs) at 12-week-old. To confirm the role of ROS in the RVLM in BP regulation, tempol was microinjected bilaterally into the RVLM of HS and LS at 12-week-old.

Results: BP was significantly higher in HS than LS from 9-week- old. Urinary norepinephrine excretion of HS was significantly higher than that of LS at 12-week-old. TBARs level was significantly higher in the RVLM of HS than that of LS. Bilateral microinjection of tempol into the RVLM induced a greater depressor response in HS than in LS. **Conclusions:** These results suggest that high salt diet increases in ROS in the RVLM of SHR which is associated with further increases in BP, at least in part via activation of the sympathetic nervous system.

PM4-02-03 MODULATION OF RENAL FUNCTION BY SUPEROXIDE AND ITS INTERACTION WITH NITRIC OXIDE IN PRE-HYPERTENSIVE REN-2 TRANSGENIC RATS

Libor Kopkan[1], Monika Thumova[2], Zuzana Huskova[2], Zdenka Vanourkova[2], Ludek Cervenka[2], Dewan SA Majid[1]
[1]Physiology, Tulane University Health Sciences Center, New Orleans, USA, [2]Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Objective: Pre-hypertensive heterozygous transgenic rats (TGR) harboring the Ren-2 renin gene exhibit an excess of superoxide (O2-) production. We evaluated the hypothesis that enhanced O2- activity and its interaction with nitric oxide (NO) is involved in the development of hypertension in this model. **Methods:** Renal responses to acute infusion of O2- scavenger, tempol (150 μg/min/100g BW), and NO synthase inhibitor, nitro-L-arginine methylester (L-NAME, 5 μg/min/100g) directly into the renal artery were evaluated in anesthetized male TGR and compared with those in Hanover-Sprague Dawley rats (HanSD). Inulin and PAH clearances were used to determine glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. 8-isoprostane concentration in urine was determined by enzyme immunoassay. **Results:** Although arterial pressure and basal renal function were not different in pre-hypertensive TGR and HanSD rats, infusion of tempol alone caused significant increases in GFR and RPF (12±2 and 10±4 %, respectively) in TGR (n=9) but not in HanSD (n=9). Compared to HanSD, 8-isoprostane excretion was significantly higher in TGR (41%) which was attenuated during tempol treatment. L-NAME infusion caused greater decreases in GFR and RPF in TGR (-22±4 and -34±4 %; n=10) than in HanSD rats (-10±4 and -19±3 %; n=10). In TGR (n=12), greater hemodynamic effects of L-NAME were attenuated by co-infusion of tempol. **Conclusion:** These data suggest that the enhanced O2- activity and its interaction with NO modulates renal function during the pre-hypertensive phase in TGR and thus plays a role in the pathophysiology of the development of hypertension in this transgenic rat model.

PM4-02-04 IMPORTANT ROLE OF TIMP-3 IN OXIDATIVE STRESS PRODUCTION

Masayoshi Higuchi[1], Osamu Yasuda[1], Hidenobu Kawamoto[1], Toyohiko Yokoi[1], Hiromi Rakugi[1], Keisuke Fukuo[2], Toshio Ogihara[1]
[1]Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Japan, [2]Department of Food Sciences & Nutrition, School of Human Environmental Sciences, Mukogawa Women's University, Japan

Objectives: Tissue inhibitor of metalloproteinase-3 (Timp-3) is a multifunctional protein, originally cloned as an inhibitor of matrix metalloproteinases (MMP). This study was performed to investigate the role of Timp-3 in oxidative stress production in murine vascular smooth muscle cells (VSMC). **Methods:** VSMC were cultured from the aortas of wild-type and Timp-3 knockout mice. After the stimulation by angiotensin II (AII), oxidative stress production in cultured VSMC was analyzed by dihydroethidium (DHE) staining, and expression of Timp-3 and angiotensin converting enzyme (ACE) was examined by Western blot analysis. MMP acivity in cultured medium of VSMC was measured by gelatin zymography. Timp-3 expression was restored by the transfection of an expression vector. Wild-type and Timp-3 knockout mice were fed with drinking water containing L-NAME, and ACE expression in cardiac microvessels was examined by immunostaining. **Results:** Timp-3 expression in wild-type and its absence in Timp-3 knockout VSMC were confirmed by Western blot analysis. ACE expression was decreased in Timp-3 knockout VSMC compared to the wild-type. Gelatin zymography revealed enhanced activity of MMP2 in the medium of Timp-3 knockout VSMC. In DHE analysis, oxidative stress production in AII-stimulated VSMC was markedly reduced by Timp-3 deficiency. This decrease was restored by Timp-3 transfection. Immunohistochemistry of cardiac microvessel in wild-type mice revealed that L-NAME induces ACE expression and oxidative stress, both of which were decreased in Timp-3 knockout mice. **Conclusions:** It is suggested that Timp-3 play roles in AII-induced oxidative stress production in VSMC.

PM4-02-05 CHRONIC OR ACUTE INTERACTION OF ANGIOTENSIN II ON OXIDATIVE STRESS PRODUCTION BY GLUCOSE PRETREATED MESANGIAL CELLS

Noriko Miyazawa[1], Michiaki Abe[1], Takafumi Toyohara[1], Takehiro Suzuki[1], Kenji Nakayama[1], Akiko Ishii[2], Hiromi Shiwaku[1], Eisuke Shibata[1], Masayuki Tanemoto[1], Fumitoshi Satoh[1], Takaaki Abe[1], Sadayoshi Ito[1]
[1]Tohoku University Graduate School of Medicine, [2]Tokyo Wemen's Medical University, The Diabetic Center, Japan
michiabe@mail.tains.tohoku.ac.jp

Oxidative stress leads to glomerular mesangial changes, which are recognized in early stage of diabetic nephropathy. Previous studies showed either high concentration of D-glucose (Glu) or angiotensin II (AII) stimulated superoxide (O_2^-) production in mesangial cells. However, direct interaction of AII and Glu on O_2^- production has not been clarified. We examined whether AII and Glu synergistically interact on O_2^- production in mesangial cells. Human or rat mesangial cells were cultured on cover slips and assessed under a real-time fluorescent microscopy. In an acute study, continuous flow of 10^{-7} MAII stimulated mesangial cells pretreated with several concentrations of Glu. AII stimulated the oxidative stress, measured by an indicator of 5',6'-chloromethyl-2',7'dichlorodihydro-fluorescein diacetate, in dose-dependent manner of Glu. The changes was inhibited by RNH-6270, an AII type 1 receptor antagonist, and augmented by PD-123319, a type 2 receptor antagonist. For chronic study, rate of O_2^- generation was determined by increases in the intensity of ethidium converted from preloaded dihydroethidium. When the cells were preincubated with AII and 13.5mM Glu, O_2^- production was higher than vehicle. However, either AII or Glu alone had no effect. Influence by osmolality was ruled out by L-glucose. TheO_2^- production was significantly inhibited by apocynin, an NADPH oxidase inhibitor, by RNH-6270, by thenoyltrifluoroacetone, a mitochondrial complex II inhibitor, or by carbonylcyanide m-chlorophenylhydrazone, an uncoupler of mitochondrial membrane proton gradient. These data suggests that mesangial cell received synergistic oxidative stress by AII and Glu via NADPH oxidase and mitochondrial pathway.

PM4-02-07 OXIDATIVE STRESS-ASSOCIATED VASCULAR AGING IS XANTHINE OXIDASE BUT NOT NAD(P)H OXIDASE DEPENDENT.

Mohammad Newaz, Adebayo Oyekan
Center for Cardiovascular Diseases, Texas Southern University, Housto, Texas. USA
Newaz_ma@tsu.edu

Aging is an independent risk factor for the development of cardiovascular disease. Vascular aging is characterized by endothelial dysfunction that is primarily attributed to increased superoxide formation. To date, the source of this age-associated increased superoxide production remains ambiguous. However, NAD(P)H oxidase pathway was excluded. In this study, we tested the hypothesis that xanthine oxidase is the source of superoxide generation and impaired vascular function in aging. Male Sprague Dawley rats, 4 months (Young) and 18 months (Aged) old, were compared for systolic blood pressure (SBP), endothelial function, and free radical generation via NAD(P)H oxidase and xanthine oxidase. Mean SBP was higher (36±2%) in the aged group compared to the young rats. This was accompanied by a reduced acetylcholine-induced renal vasodilatation as determined by isolated perfused kidney preparation. Urinary excretion of nitrite was lower in the aged rats and this was associated with a reduced expression of eNOS and iNOS proteins in the aorta. Aged rats showed a ~2-fold increase in free radical generation as evident by increased plasma 8-Isoprostane level and a 3-fold increase in proteinuria compared to the young rats. Vascular NADP(H) oxidase activity was unchanged between both groups. Similarly, expression of p67phox and p47phox component of NAD(P)H oxidase was unchanged. However, xanthine oxidase activity was increased by 19±1% in the aged rats accompanied by an increased xanthine oxidase protein expression in the aortic homogenate. These results suggest that increased free radical generation-associated increase in SBP in aged rats is xanthine oxidase but not NAD(P)H oxidase-dependent.

PO4-116 DIASTOLIC FUNCTION AT REST AND DURING DOBUTAMINE STRESS ECHOCARDIOGRAPHY IN PATIENTS WITH HYPERTENSION COMPARED WITH ASSESSMENT OF CORONARY MICROCIRCULATION BY CARDIOVASCULAR MAGNETIC RESONANCE

Danuta Czarnecka[1], Agnieszka Kwiecien-Sobstel[1], Agnieszka Olszanecka[1], Mieczyslaw Pasowicz[2], Piotr Klimeczek[2], Robert Banys[2], Artur KLecha[1], Kalina Kawecka-Jaszcz[1]
[1]Jagiellonian University, Poland, [2]John-Paul II hospital, Cracow

The aim of the study was to asses left ventricle diastolic function in patients with arterial hypertension AH and to compare the results with the parameters of myocardial perfusion detected by cardiovascular magnetic resonance imaging CMR. The study included 76 patients: 54 women and 22 men: mean age 55.6 ± 7.8 years, with AH and normal coronary angiograms. Echocardiographic examination was performed to evaluate left ventricular diastolic dysfunction LVDD defined as IVRT < 90 msek. Each patient underwent dobutamine stress echocardiography. Using pulsed wave tissue Doppler echocardiography TDE peak early E´ and late A&acute velocities of basal segments of intraventricular septum IVS and lateral wall LW were measured at rest and high dose of dobutamine. In all subjects myocardial first pass perfusion CMR imaging, both at rest and during an infusion of adenosine was performed. Quantitative perfusion analysis was performed to derive the myocardial perfusion reserve index MPRI. Results: Patients were divided into two groups: with LVDD: n=41 and without LVDD: n=35. Patients with LVDD had significantly lower E´ velocity: 6.57 ±1,8 vs 9.1± 2.3, p=0.0003 and E´/A´ ratio: 0.57 ± 0.22 vs 0.83 ± 0.36, p=0.003, but only for IVS and during high dose of dobutamine. Neither at rest, nor during stress we could observe any differences in MPRI between patients with and without LVDD. Conclusion: In the examined patients with AH, the presence of LVDD influenced the pulsed wave TDI parameters during dobutamine stress echocardiography for IVS, and did not affect coronary microcirculation assessed by perfusion CMR.

PO4-117 THE SYMPATHO-INHIBITORY EFFECT OF STATIN IN THE SHRSP ALONG WITH THE ANTI-OXIDANT EFFECT IN THE RVLM

Takuya Kishi[1], Yoshitaka Hirooka[1], Akira Takeshita[2], Kenji Sunagawa[1]
[1]Kyushu University Graduate School of Medical Sciences, [2]Aso-Iizuka Hospital, Japan

tkishi@cardiol.med.kyushu-u.ac.jp

Statins have reported to increase endothelial nitric oxide synthase (eNOS) expression and activity independent of cholesterol-lowering effects. Recently, we demonstrated that atorvastatin had sympatho-inhibitory effects with upregulation of eNOS in the brain in stroke-prone spontaneously hypertensive rats (SHRSP). Furthermore, we also demonstrated that reactive oxygen species (ROS) in the rostral ventrolateral medulla (RVLM) of the brain stem, where the vasomotor center is located, was significantly increased in SHRSP and that ROS in the RVLM contribute to sympatho-excitatory effects in SHRSP. Therefore, the aim of the present study was to determine whether atorvastatin reduces oxidative stress in the RVLM of SHRSP associated with sympatho-inhibitory effects. SHRSP and Wistar-Kyoto (WKY) rats were treated with or without atorvastatin (50mg/kg per day) for 30 days. Systolic blood pressure (SBP) and heart rate were measured using the tail-cuff method. As the parameter of the sympathetic nerve activity, urinary norepinephrine excretion (UNE) was measured for 24 hours before and after the treatment. Treatment with atorvastatin significantly decreased SBP and UNE in SHRSP, but not in WKY (199±6 vs 173±5 mmHg, 1.41±0.05 vs 1.07±0.04 μg, p<0.05, n=5). Thiobarbituric acid-reactive substances (TBARS) levels in the RVLM tissue obtained using the punch-out technique were used as measures of oxidative stress. Treatment with atorvastatin decreased TBARS levels in the RVLM of SHRSP (0.75±0.04 vs 0.57±0.03 μmol/g wet wt, p<0.05, n=5), but not in WKY. These results suggest that atorvastatin reduces oxidative stress in the RVLM of SHRSP, which might contribute to the sympatho-inhibitory effects of atorvastatin in SHRSP.

PO4-118 SESAME OIL REDUCES BLOOD PRESSURE IN MILD HYPERTENSIVE PATIENTS

Ganapathy Sambandam[1,2,3,4], Devarajan Sankar[2], Ramakrishna Rao[3], Viswanathan Pugalendi[4]
[1]Prof.Maniarasan Memorial Poly Clinic, Chidambaram-608 001, Tamilnadu, India, [2]Department of Biochemistry, Annamalai University,Annamalainagar-608 002, Tamilnadu, India, [3]Faculty of Medicine, Rajah Muthiah Medical College and Hospital,Annamalai University, Annamalainagar-608 002, Tamilnadu, India., [4]Department of Biochemistry, Annamalai University,Annamalainagar-608 002, Tamilnadu, India

sambandamg@sify.com

The objective of this study was to evaluate the effect of sesame oil as sole edible oil in mild hypertensive patients, since the oil exerted significant reduction in blood pressure in drug-taking hypertensive patients. Twenty-five patients aged 40-50 years of both the sexes with mild hypertension participated in this study. Sesame oil (Idhayam Gingelly oil) was supplied to the patients and instructed to use in place of other edible oils for 60 days. The patients were advised to consume approximately 35 g of oil per day as cooking medium. Clinical follow up and biochemical determinations such as plasma lipid profile, electrolytes lipid peroxidation, enzymic and non enzymic antioxidants were measured at baseline and after 60 days of sesame oil substitution. Blood pressure was measured at every 15th day during the study period. Blood pressure and diastolic blood pressure remarkably reduced upon sesame oil substitution. Total cholesterol, low-density lipoprotein cholesterol and triglycerides were significantly reduced while significant elevation was noted in high-density lipoprotein cholesterol. Plasma levels of sodium reduced significantly while potassium elevated. Lipid peroxidation decreased significantly. Activities of superoxide dismutase, catalase and glutathione peroxidase and the levels of vitamin C,vitamin E, beta carotene and reduced glutathione were increased significantly upon the substitution of sesame oil. Sesame oil as sole cooking oil reduced blood pressure in mild hypertensive patients and influenced beneficially the levels of lipid profile, electrolytes, lipid peroxidation and antioxidants.

PO4-119 IMPACT OF EDIBLE OILS ON HYPERTENSION MANAGEMENT

Devarajan Sankar[1,2,3,4], Ganapathy Sambandam[2], Ramakrishna Rao[3], Viswanathan Pugalendi[4]
[1]Prof.M.M.Poly Clinic,Chidambaram-608 002, Tamilnadu, India, [2]Prof. M.M.Poly Clinic,Chidambaram-608 002, Tamilnadu, India, [3]Rajah Muthiah Institute of Health Sciences, Annamalai University, Annamalainagar-608 002, Tamilnadu, India, [4]Department of Biotechnology, Aarupadai Veedu Institute of Technology, Vinayaka Mission's Research Foundation Deemed University, Paiyanoor-603 104, Chennai, Tamilnadu, India

devsankara@yahoo.co.in

Objective: To evaluate the role of edible oils on blood pressure, lipids and redox status in drug-taking hypertensive patients Design and Methods: Five hundred and seventy hypertensive patients medicated with nifedipine were divided into four groups (356 patients-sesame oil; 87 patients-sunflower oil; 47 patients-groundnut oil and 40 patients-palm oil). The control group (n=40) received only the drug. The respective oils were supplied to the patients and instructed to use as the only edible oil for 60 days. Blood pressure, lipids, lipid peroxidation, enzymic and non-enzymic antioxidants were measured at baseline (0 day) and after 60 days of oil substitution.Results: Patients with nifedipine alone or with respective oils had lowered blood pressure differently. Sesame oil substitution significantly lowered the need of medication. Plasma and RBC concentration of saturated fatty acids reduced while mono and polyunsaturated fatty acids elevated and the vicissitude is more in sesame oil group. TC, LDL-C and TG decreased while HDL-C elevated in sesame and sunflower oil. Elevation of HDL-C and TG was noted in groundnut and palm oil. Pronounced reduction of lipid peroxidation was noted in sesame oil. Activities of superoxide dismutase elevated in all the groups whereas glutathione peroxidase and catalase increased only in sesame oil. Vitamin C, vitamin E, beta carotene and reduced glutathione increased in sesame oil group whereas elevation of vitamin E and beta carotene noted only in sunflower and groundnut oil. Conclusions: Among the four oils, sesame oil offers better protection over blood pressure lipid composition, lipid peroxidation and antioxidants.

ISH 2006

ISH2006
Investigator Initiated Symposium

Central Cardiovascular Regulation: From Hypertension to Heart Failure

Abstract

Date & Time: October 14, 2006 13:30-18:35
Place: Hotel Okura Fukuoka (4F Heian 1)

ISH2006 Investigator Initiated Symposium
Central Cardiovascular Regulation: From Hypertension to Heart Failure

October 14, 2006 Hotel Okura Fukuoka
Organizers: John Chalmers *Sydney, Australia*
Yoshitaka Hirooka *Fukuoka, Japan*

Program

Moderators: John Chalmers Yoshitaka Hirooka

13:30-13:40 **Opening Remark and Introduction**
Central cardiovascular regulation: From hypertension to heart failure
Yoshitaka Hirooka (Fukuoka, Japan)

13:40-14:10 **State of the Art Lecture**
CNS effects of aldosterone in hypertension and heart failure
Frans HH Leenen (Ontario, Canada)

14:10-14:15 Q&A

14:15-14:35 Effects of angiotensin II and angiotensin II receptor blockers (ARBs) on peripheral and central sympathetic nervous system
Hiroo Kumagai (Tokyo, Japan)

14:35-14:40 Q&A

14:40-15:00 Decoding the neurotransmitter systems that control the cardiovascular system: From molecular to clinical
Paul M. Pilowsky (Sydney, Australia)

15:00-15:05 Q&A

15:05-15:25 Mitogen-activated protein kinases as novel signaling pathways in angiotensin II - induced hypertension
Julie Y.H.Chan (Kaohsiung, Taiwan)

15:25-15:30 Q&A

15:30-15:55 *Coffee Break*

Moderators: Kaushik P. Patel Yoshitaka Hirooka

15:55-16:15 Hypothalamic mechanisms regulating the cardiovascular response to stress: Role of 5-HT1a receptors
Roger Dampney (Sydney, Australia)

16:15-16:20 Q&A

16:20-16:40 Central mediators of sympatho-excitation in heart failure: The angiotensin-ROS-nitric oxide connection
Irving H. Zucker (Omaha, USA)

16:40-16:45 Q&A

16:45-17:05 Role of interventional autonomic control in the management of heart failure
Masaru Sugimachi (Osaka, Japan)

17:05-17:10 Q&A

17:10-17:30 Combined influence in essential hypertension pathogenesis of increased single fibre sympathetic nerve firing rates and epigenetic silencing of the noradrenaline transporter gene
Murray Esler (Melbourne, Australia)

17:30-17:35 Q&A

17:35-17:55 Sympathetic and cardiovascular actions of leptin and the concept of selective leptin resistance: Implications for obesity-induced hypertension
Allyn L. Mark (Iowa, USA)

17:55-18:00 Q&A

18:00-18:20 **Special Lecture**
Blood pressure and stroke prevention
John Chalmers (Sydney, Australia)

18:20-18:25 Q&A

18:25-18:35 **Closing Remark**
John Chalmers (Sydney, Australia)

Reception to follow

ISH 2006

ISH2006 Investigator Initiated Symposium

Central Cardiovascular Regulation: From Hypertension to Heart Failure

John Chalmers, MD, PhD
Senior Director and Head of Research Advisory Unit,
The George Institute for International Health
University of Sydney

Yoshitaka Hirooka, MD, PhD
Department of Cardiovascular Medicine
Kyushu University Graduate School
of Medical Sciences

Organizers of the symposium

Greeting from Organizers:

Dear Colleague,

We are pleased to announce an Investigator-Initiated Symposium on "Central Cardiovascular Regulation: From Hypertension to Heart Failure" at the 21st International Society of Hypertension meeting in Fukuoka, Japan, on Saturday, October 14, 2006 just before the main meeting. This symposium will focus on neural control of circulation in hypertension and heart failure ranging from basic to clinical points of view. The wide range of conceptual and technological innovations in this fast moving field should make the Symposium attractive to investigators from diverse backgrounds, in the USA, Europe, and Asian Pacific. In addition, the symposium will provide participants an opportunity to get together, discuss scientific issues, and build closer relationships and collaborations.

The program consists of presentations from many of the most active investigators in this field and we will welcome you as a discussant. We hope that many of our colleagues apart from the invited speakers, will participate in the symposium. All registrants at the 21st ISH Congress will be welcome to participate.

We cordially invite you to be an active participant in this unique symposium and look forward to seeing you in Fukuoka.

Sincerely,

Central cardiovascular regulation: From hypertension to heart failure

Yoshitaka Hirooka
Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

Hypertension remains a major risk factor for cardiovascular events compared to other risk factors. Hypertension causes cardiac hypertrophy, ischemic heart disease, and eventually leads to heart failure, which is the final condition that results from many structural heart diseases. Although the etiology of essential hypertension is multifactorial, it is due to abnormal regulatory systems. The major regulatory systems of blood pressure and body fluid homeostasis are the sympathetic nervous system and the renin-angiotensin system. The factors that modify these two major regulatory systems and to what extent they modify these systems, are important research questions. Recent studies indicate that hypertension has a large neural component. In heart failure, it is now clear that an enhanced sympathetic drive contributes to the development of the disease and leads to a poor prognosis. Recent large clinical trials indicate that ß-blockers are effective in patients with heart failure.

Sympathetic nerve activity is determined by the cardiovascular center in the brainstem. The hypothalamus and other areas in the brain influence the cardiovascular center, thereby determining the basal sympathetic tone. The baroreceptor reflex is the major regulatory system for maintaining blood pressure at a normal level. In the hypertensive state, this reflex is reset to a higher pressure level, which is considered to be the major reason for hypertension. It is difficult, however, to explain the mechanism(s) by which hypertension persists and progresses over time with the peripheral mechanism(s) alone. Therefore, many investigators of the neural control of circulation are now interested in central cardiovascular regulation. In particular, Drs. Donald Reis, Paul Korner, and Michael Brody are the leaders in initiating this field of research. Today, following their lead, investigators are using modern technology to increase our knowledge in this field. A series of studies from our laboratory demonstrated that nitric oxide, reactive oxygen species, and Rho-kinase are significantly involved in the neural mechanism(s) of hypertension and heart failure. Many questions remain, however, regarding their mechanistic and pathophysiological significance. In this symposium, authorities in this field will provide insight into the many aspects of hypertension and heart failure from molecular approaches to integrative physiology, and from basic to clinical studies.

I am very happy that this Investigator-Initiated Symposium for ISH2006 is in Fukuoka. I thank Professor Chalmers, the co-organizer, and the organizing committee of ISH2006. I hope that this symposium will be a great opportunity for many investigators from all over the world in this field and will encourage young scientists to continue their research with confidence in the future!

References

1) Kishi T, Hirooka Y, et al. Hypertension 39: 264-268, 2002.
2) Ito K, Hirooka Y, et al. Circ Res 92: 1337-1343, 2003.
3) Kishi T, Hirooka Y, et al. Circulation 109: 2357-2362, 2004.
4) Kimura Y, Hirooka Y, et al. Circ Res 96: 252-260, 2005.
5) Sakai K, Hirooka Y, et al. Am J Physiol Heart Circ Physiol 289: H2159-H2166, 2005.

Vol 114, No 18, October 31, 2006
ISSN 0009-7322
http://circ.ahajournals.org

American Heart Association®
Learn and Live℠

SUPPLEMENT TO

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Abstracts from

scientific sessions 2006

basic science
clinical science
population science

Sessions: November 12–15
Exhibits: November 12–14
Chicago, Illinois

scientificsessions.org

Named and Invited Lectures 2006

2006 Russell Ross Memorial Lectureship in Vascular Biology • 2006 George L. Duff Memorial Lecture • 2006 Sol Sherry Distinguished Lecture in Thrombosis • 2006 Thomas W. Smith Memorial Lecture • 2006 George E. Brown Memorial Lecture • 2006 Dickinson W. Richards Memorial Lecture • 2006 Katharine A. Lembright Award • 2006 William W. L. Glenn Lecture • 2006 William J. Rashkind Memorial Lecture • 2006 Helen B. Taussig Memorial Lecture • 2006 Charles T. Dotter Memorial Lecture • 2006 Laennec Clinician/Educator Lecture • 2006 James B. Herrick Award and Lecture • 2006 Ancel Keys Memorial Lecture • 2006 Lewis K. Dahl Memorial Lecture • 2006 Donald Seldin Lecture • 2006 Robert Levy Memorial Lecture • 2006 Stroke Council Award and Lecture • 2006 Distinguished Scientist Lecture

Council Early Career Investigator Awards 2006

Lewis N. and Arnold M. Katz Basic Science Research Prize for Young Investigators • Melvin L. Marcus Young Investigator Award in Cardiovascular Science • Cournand and Comroe Young Investigators Prize in Cardiopulmonary and Critical Care • Melvin Judkins Young Investigator Award in Cardiovascular Radiology • Vivien Thomas Young Investigator Award • Samuel A. Levine Young Clinical Investigator Award • Elizabeth Barrett-Connor Research Award for Young Investigators in Training • Martha N. Hill New Investigator Award • Laennec Society Young Clinician Award • NPAM New Investigator Award

Abstracts From the Scientific Sessions 2006