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THE EFFECTS OF ENERGY AND/OR SALT RESTRICTION OF HYPERTENSION AND KIDNEY INJURY IN OBESE SALT-SENSITIVE HYPERTENSIVE RATS

Katsuyuki Ando, Megumi Fujita, Masaomi Nangaku
Department of Nephrology and Endocrinology, University of Tokyo Graduate School of Medicine, Tokyo, Japan

To clarify the role of obesity and salt excess in hypertension during young age, we examined the effects of energy- and/or salt-restricted diets in hypertension and kidney injury of DahlS.Z-Lepr^{fa}/Slc (DS/fa) rats, which were produced by crossbreeding Zucker obese (ZO) and Dahl salt-sensitive (DS) rats. Firstly, we examined the characteristics of DS/fa rats, comparing blood pressure (BP), kidney function, metabolic parameters, and plasma renin activity (PRA), aldosterone concentration (PAC) and weight of adrenal glands. And then, the effects of energy- (60 % restriction compared to *ad libitum* feeding) and/or salt- (0.05 % salt) restricted diets on the above-mentioned parameters in DS/fa rats. Body weight gain was almost similar between DS/fa and ZO rats, which was extremely greater than body weight of DS rats. Systolic BP was higher in DS/fa rats compared to DS and ZO rats. Fasting blood sugar (BS) and insulin (IRI) was similarly higher in DS/fa and ZO rats than DS rats. On the other hand, dyslipidemia was accelerated in DS/fa rats than ZO rats, which showed higher serum total cholesterol (TC) and triglyceride (TG) than DS rats. Especially, serum TG was extremely greater in DS/fa rats. Urinary protein and blood urea nitrogen (BUN) were increased in DS/fa rats. Also, renal histological damage was progressed in DS/fa rats. PAC was increased in ZO rats compared to DS rats but it was similar between DS/fa and DS rats. Compared to DS rats, PRA was increased in ZO rats but suppressed in DS/fa rats. As a result, PAC/PRA ratio was increased in DS/fa rats but almost same between ZO and DS rats. Weight of adrenal glands was greater in DS/fa rats than ZO and DS rats. Thus, aldosterone production may be enhanced in DS/fa rats: In fact, serum potassium was decreased in DS/fa rats. Energy restriction decreased body weight of DS/fa rats but salt restriction did not. Also, Energy restriction decreased systolic BP but salt restriction only marginally did. Glucose metabolism was ameliorated by energy restriction but not by salt restriction. However, dyslipidemia, especially serum TG, was greatly improved by all dietary treatments, although the anti-dyslipidemic effects were strengthened with energy restriction than with salt restriction. Urinary protein was also decreased with all diets, which were again greater with energy restriction. In addition, both energy- and salt restriction further suppressed urinary protein. BUN was similarly normalized by all diets. PAC/PRA ratio and weight of adrenal glands were also decreased with all diets. In conclusion, DS/fa rats showed hypertension and kidney injury from young age, due to extreme obesity and salt retention. Increased aldosterone production might be one of the possible common mechanisms that obesity and salt excess progress hypertension and renal injury.

RENAL SYMPATHETIC ACTIVITY AND RENAL CORTICAL AND MEDULLARY BLOOD FLOW DISTRIBUTION IN SHRSP

Takuzo Hano, Naomi Iwane, Noriko Miyoshi, Yumi Koike, Masato Mizukoshi
Graduate School of Medicine, Medical Education and Population-based Medicine, Wakayama Medical University, Wakayama, Japan

Aim of present study was to clarify the difference of renal cortical and medullary blood flow distribution by renal nerve stimulation between spontaneously hypertensive rats (SHRSP) and WKY and the contribution of nitric oxide to altered blood flow distribution in SHRSP. We used 6-7 and 16-17 week old SHRSP and WKY as control rats. Renal nerve around left renal artery was stimulated by 2, 5, 10 and 15 Hz under the anesthesia of isoflurane. Renal blood flow was measured using laser tissue blood flow meter with a noncontact probe for cortical blood flow and a contact glass-fiber needle type of probe for medullary blood flow. Arterial blood pressure was monitored using pressure transducer. After a series of electrical stimulation, 1 mg of LNAME was injected and same protocol was repeated. In young WKY, low frequency of electrical stimulation caused a slight increase of blood flow and mass in both renal cortex and medulla. High frequency stimulation caused a decrease of cortical blood flow in young and aged WKY. Blood flow in medulla showed no significant change by electrical stimulation. In contrast to blood flow, blood mass of renal cortex was significantly increased by electrical stimulation. Renal hemodynamic changes of aged WKY were similar to those of young WKY. Young SHRSP showed a decrease of cortical and medullary blood flow and a marked increase of cortical blood mass. Cortical blood flow was reduced and blood mass was increased in aged SHRSP. Reduction of medullary blood flow of aged SHRSP was more than that of young SHRSP. LNAME abolished the transient increase of cortical blood flow by electrical stimulation in all rats. NAME augmented the reduction of medullary blood flow by electrical stimulation in aged WKY. LNAME suppressed the increase of cortical blood mass completely and augmented medullary blood flow reduction in young SHRSP. In contrast to young SHRSP, LNAME showed no effects on blood flow reduction by electrical stimulation in renal medulla of aged SHRSP. These results suggest that cortical blood flow is reduced and blood mass is increased by renal sympathetic nerve activation and cortical blood is shifted to medulla. These mechanisms can protect the blood flow reduction in renal medulla through the nitric oxide production. Renal sympathetic nerve activation causes a reduction of cortical blood flow which is partially counteracted by the production of nitric oxide in SHRSP. Medullary blood flow is reduced especially in aged SHRSP and this phenomenon might be related to the reduction of nitric oxide synthesis.

CHORNIC TREATMENT OF TAURINE CAN IMPROVE ALTERED RENAL CORTICAL AND MEDULLARY BLOOD FLOW DISTRIBUTION IN SHRSP

Takuzo Hano, Naomi Iwane, Noriko Miyoshi, Yumi Koike, Masato Mizukoshi
Graduate School of Medicine, Medical Education and Population-based Medicine, Wakayama Medical University, Wakayama, Japan

In the previous experiments, we showed that high frequency stimulation of renal nerve caused a transient increase of cortical blood flow and a sustained decrease of blood flow in WKY. In contrast, blood flow in renal medulla showed no significant changes. Young SHRSP showed a decrease of cortical and medullary blood flow by renal nerve stimulation. The magnitude of cortical blood flow reduction in aged SHPSP was similar to young SHRSP and that of medullary blood flow reduction was more than that in young SHRSP. LNAME augmented the reduction of medullary blood flow by electrical stimulation in aged WKY and young SHRSP. LNAME showed no effects on blood flow reduction in medulla in aged SHRSP. These results indicate that medullary blood flow is protected through the nitric oxide production in normotensive rats and this mechanism is blunted in hypertensive rats. Reduction of nitric oxide production in renal medulla might be contributed to this phenomenon. Aim of this study was to clarify that chronic treatment of taurine can improve the altered renal cortical and medullary blood flow distribution by renal nerve stimulation in SHRSP. We used 16-17 weeks old SHRSP/Izm which were divided to two groups, taurine group and control group. Three percent of taurine was given to taurine group for 3 weeks and tap-water was given to control group. Renal nerve around left renal artery was stimulated by 2, 5, 10 and 15 Hz under the anesthesia of isoflurane. Renal blood flow was measured using laser tissue flow meter with noncontact probe for cortical blood flow and contact glass-fiber needle type of probe for medullary blood flow. After a series of electrical stimulation, 1 mg of LNAME was injected and same protocol was repeated. Renal blood flow distribution was similar to the previous study. Cortical and medullary blood flow in aged SHRSP was reduced by electrical stimulation. Chronic treatment of taurine suppressed the reduction of blood flow by electrical stimulation in renal medulla but not in renal cortex of aged SHRSP. Injection of LNAME showed no effects on renal blood flow distribution in aged SHRSP. In conclusion taurine can improve the reduction of renal medullary blood flow in aged SHRSP but this phenomenon was not counteracted by the treatment of LNAME. Mechanism of the improvement of renal medullary blood flow by taurine is unclear but the suppressive effect of taurine on sympathetic nerve activity as shown in our previous data might be related to these mechanisms.

ACTIVATION OF THE CALDIAC HYPERTOPHY-RELATED CALCINEURIN BY CALPAIN IN THE SPONTANEOUSLY HYPERTENSIVE RATS

Ayami Hasegawa[1], Hisayuki Inuyama[1], Yukiko Yamamoto[2], Kazuhiko Yamamoto[1]
[1]Cluster of Biotechnology and Chemistry, Graduate School of Systems Engineering, Kinki University, Higashi-Hiroshima, Japan, [2]Life Science Institute of Kinki University, Osaka-Sayama, Japan

Metabolic syndrome patients had accumulation of the visceral fat, and prone to high blood pressure, dyslipidemia or diabetes. Morbidities of these diseases rise with the aging, and besides many patients have been under therapy, there are much more people suspected of suffering those diseases. It has become serious economic and social problems, in not only developed countries and in the developing countries. One of the main causes of the heart diseases and the cerebro- vascular diseases are the high blood pressure. The mortality of the ischemic diseases followed to a cancer, and that prominent symptom arteriosclerosis lead to the cardiac hypertrophy, finally cause the heart failure, the end-stage of chronic cardiopathy. To establish the preventive method and the development of the cure, elucidation of the molecular mechanism of the onset of those diseases is required, and our objective of our study is to elucidate mechanisms of cardiac hypertrophy. It is known that many calcium dependent intracellular signaling factors are pertinent to the cardiomyocyte remodeling, shrinkage imperfection, cell death and hypertrophy. Calcineurin (CN) is a Ca^{2+} -dependent serine/threonine phosphatase and composed from CnA- and CnB-subunit, which subunits have catalytic and activation domain, respectively. CnA is activated by assemble of it and Ca^{2+} bound -CnB and -calmodulin (CaM). Transcription factor NFAT is dephosphorylated by activated calcineurin at the cytosol and transported into the nucleus, and enhancing cardiac hypertrophy-related genetic transcription. Calpain (CAPN) is a Ca^{2+} -dependent proteolytic enzyme in the cytoplasm concerning diverse protein activation or degradation processes. It was reported that CAPN was activated by the elevation of the intraneural cell Ca^{2+} concentration in schizophrenia patient, and then CnA will be converted to constitutively active form by limited- proteolysis by CAPN and caused neural cell impairing. It may be trigger in cardiac hypertrophy, like the irreversible activation of CN by CAPN occurred in neural cell, but it is not obviously now. In this study, we intend to confirm possibility of the onset of hypertrophy *via* activation of CN by CAPN. Expression levels of genes (CnA, CnB and CAPNs) of the hypertensive rats (SHR/kpo, SHRSP/kpo and M-SHRSP/kpo) and normotensive rats (WKY/kpo) were measured by RT-PCR methods. In the heart of SHRs, the expression pattern of catalytic subunit CnA was correlated to the blood pressure. As one of etiologies of the cardiac hypertrophy is the high blood pressure, we can predict that the expression of CN is higher in M-SHRSP than that in other SHRs. But our results showed the level of the activation subunit CnB of M-SHRSP/kpo was lower than other SHR/kpo and WKY/kpo. The decreases of CnB expression level suppress the dephosphorylation activity of CN, and as result regulate the transcriptional activity of NFAT. The cardiac dilatation was observed frequently in SHRSP/kpo, but was not so in M-SHRSP/kpo. These results show that CnB serves as regulator of the cardiac hypertrophy caused by the Ca^{2+}-calcineurin system. It is reported that there are more than 12 calpains in the mammalian, CAPN1 exists in all tissues. Expression of CAPN1 was highest in the heart of SHRSP/kpo compared to that in SHRs. Suggesting the possibility of activation of CN by CAPN and the relevance to the cardiac hypertrophy *via* it. It is reported that CAPN3 is anchored to the sarcomere and be related to limb-girdle muscular dystrophy, but overexpression of CAPN3 represented no phenotype. Expressions of CAPN3 of heart of hypertensive SHRs are same levels among them, but are higher than that of the normotensive WKY/kpo. Increase of CAPN3 may be the compensatory effect on the load of the heart muscle due to increased blood pressure. In hypertension, such as chronic renal failure has become a problem. It is conceivable that CN caused the remodeling of cells and related to damage of kidney and heart. Levels of CN and CAPN of kidney of WKY/kpo were trace, but those of CnA and CnB were high in SHR/kpo and M-SHRSP/kpo. And expression of CAPN of kidney of SHR/kpo is high and of M-SHRSP/kpo was a little. As CN of SHR/kpo kidney will be activated by CAPN and CnB, and in M-SHRSP/kpo, it was only by the latter pathway, activation mechanisms of CN in kidney differed from that of the heart. These result show it is useful for study each molecular mechanisms as tissues individually to elucidate etiology of diseases.

TRANSLATIONAL RESEARCH BASED ON THE ANALYSES OF CELL-SPECIFIC (PRO) RENIN RECEPTOR KNOCKOUT MICE

Atsuhiro Ichihara
Department of Medicine II, Endocrinology and Hypertension, Tokyo Women's Medical University, Tokyo, Japan

Prorenin generated from renin gene is an inactive precursor of renin and secretes into the blood freely. Ten percentages of prorenin generated in juxtaglomerular cells are processed to active renin in the secretory granules and thereafter secrete into the blood. Consequently, plasma concentrations of prorenin are nine times higher than those of renin. In 2002, (pro)renin receptor [(P)RR] is identified as one-transmembrane protein from human kidneys. The (P)RR binds prorenin and renin in tissues, not only leading to their activation, but also causing intracellular MAPK signaling. As a key player in the tissue renin-angiotensin system (RAS), (P)RR activation plays an important role in the development of end-organ damage in the RAS dependent diseases such as hypertension and diabetes. We developed the cell-specific (P)RR knockout mice to elucidate physiological roles of (P)RR in cardiomyocytes, glomerular epithelial cells, and vascular smooth muscle cells. (P)RR includes the domain, consisting of transmembrane part and C-terminal part, called ATP6AP2 because it has been shown to be associated with vacuolar H^+-ATPase (V-ATPase). The V-ATPase is a multi-subunit proton pump involved in diverse and fundamental cellular physiology, including receptor-mediated endocytosis and recycle, processing of proteins and signaling molecules, membrane sorting and trafficking, and activation of lysosomal/autophagosomal enzymes. Recent studies using the cell-specific (P)RR knockout mice showed that (P)RR is essential for the formation and activity of the V-ATPase. Furthermore, the novel function of the (P)RR as an adaptor protein between the Wnt receptor complex and the V-ATPase was reported. Based on these findings, the (P)RR is a multi-functional molecule that regulates the tissue RAS under pathological conditions and contributes to the development, growth, and maintenance of cells under physiological conditions. Based on the insights to the (P)RR as a modulator of the tissue RAS and V-ATPase, translational research for developing a new diagnostic marker and novel therapy has been ongoing.

THE METABOLIC CONTROL FOXO RELATED GENE ANALYSIS IN SPONTANEOUSLY HYPERTENSIVE RATS

Hisayuki Inuyama[1], Ayami Hasegawa[1], Suzuka Ooisi[2] , Maki Kataoka[2], Yukiko Yamamoto[3], Kazuhiko Yamamoto[1]
[1]Cluster of Biotechnology and Chemistry, Graduate School of Systems Engineering, Kinki University, Higashi-Hiroshima, Japan, [2]Department of Biotechnology and Chemistry, Kinki University School of Engineering, Higashi-Hiroshima, Japan, [3]Life Science Institute of Kinki University, Osaka-Sayama, Japan

Metabolic syndrome and cancer are the multifactorial diseases. Environmental factors are involved in the development of disease in addition to genetic factors. Diabetes, hypertension, and lipid abnormalities are risk factors of the metabolic disorder. When the patient have supervene those diseases, pathologic condition will be more severely. There are networks of the diseases through the various factors related to the onset of them. Many factors associated with diseases have important roles both in different diseases. It is conceivable that there are some key factors related to onset and supervention. There are differences of the metabolic and the intracellular signaling state between the healthy person and the patient. It is known that the inflammation, oxidative stress, and apoptosis are basis of many diseases. Post-translational modification of proteins, signal transduction proteins, transcription factors, and enzymes such as phosphatase, phosphorylase, deacetylase and acetylase, etc., affect to the physiological and pathological states of the cell. Pathogenesis of multifactorial diseases that are affected by various factors in this manner has not been clarified. Forkhead box proteins (FOXO) are a family of transcription factors that play important roles in regulation of expression of genes involved in proliferation, differentiation, apoptosis and

longevity. Sirtuin (SIRT), NAD^+-dependent deacetylase possesses important roles in regulation of many transcription factors and other proteins. Deacetylation of FOXO and histone by SIRT regulates transcriptional rates. And SIRT has been noted that involved in various diseases in addition to cancer and aging. Both FOXO and SIRT functions are related metabolic state. It was known that regulation of each function of the FOXO and SIRT are related to the metabolic state and known there are many factors effect on them and affected by them. However, there are many unclear points relevant to the SIRT and FOXO. Purpose of this study is to clarify the roles of factors related FOXO and SIRT in a disease of metabolic syndrome such as the high blood pressure. Expression levels of genes of the hypertensive rats (SHR/kpo, SHRSP/kpo and M-SHRSP/kpo) and normotensive rats (WKY/kpo) were measured by RT-PCR methods. Although expression of FOXO1 gene of lung is correlated to the blood pressure levels of rats, the levels of liver are almost constant. As the expression patterns of FOXO family genes were resemble, the regulation mechanisms of FOXO family may be the common ways. Nuclear localization of FOXO is inhibited by phosphorylation by AKT. Expression patterns of AKT were resembled to that of FOXO. Effects on transcription activity of phosphorylation and deacetylation by AKT and SIRT of a FOXO molecule are still unclear. Further studies of FOXO related genes are needed to elucidate pathophysiological mechanism of disease.

P38 MAP KINASE MEDIATES ALDOSTERONE-INDUCED PODOCYTE INJURY IN NATRIURETIC PEPTIDE RECEPTOR (GC-A)-DEFICIENT MICE

Yukiko Kato[1], Hideki Yokoi[1], Kiyoshi Mori[1,2], Masato Kasahara[1], Yoshihisa Ogawa[1], Takashige Kuwabara[1], Hirotaka Imamaki[1], Akira Ishii[1], Kenichi Koga[1], Keita P. Mori[1], Ichiro Kishimoto[3], Akira Sugawara[1], Kazuwa Nakao[1, 2], Masashi Mukoyama[1]
[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan, [2]Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan, [3]Department of Atherosclerosis and Diabetes, National Cerebral and Cardiovascular Center, Osaka, Japan

Aldosterone plays an important role in the pathogenesis of renal injury. We have already reported that natriuretic peptide receptor/guanylyl cyclase-A (GC-A) signaling exerts renoprotective effects in subtotal nephrectomy, anti-GBM glomerulonephritis and streptozotocin-induced diabetes mouse models by eliciting natriuresis, reducing blood pressure, and inhibiting fibrosis. Furthermore, we demonstrated that uninephrectomized GC-A knockout (KO) mice with aldosterone and sodium overload exhibited accelerated hypertension with massive proteinuria. These mice exhibited increased phosphorylation of p38 MAP kinase (MAPK) mainly in podocytes. To explore the interaction between p38 MAPK and GC-A signaling, we examined the effects of p38 MAPK inhibition on renal findings of GC-A-KO mice with aldosterone administration. GC-A KO mice or wild-type mice were uninephrectomized and then fed for 4 weeks with high salt diet and infused with aldosterone (0.2 μg/kg/min) subcutaneously using an osmotic minipump. They were administered with p38 MAPK inhibitor (FR167653, 33 mg/kg/day) or hydralazine in drinking water. We examined BP, urinary albumin excretion, renal histology, electron microscopy and immunohistochemical study for nephrin, podocin and p53 which is one of downstream targets of p38 MAPK. Administration of FR167653, but not hydralazine, remarkably suppressed urinary albumin excretion by 90 % in GC-A KO mice ($p<0.01$). Glomerular hypertrophy, mesangial expansion and interstitial fibrosis were decreased in FR167653-treated aldosterone-given GC-A KO mice. In electron microscopic analysis, FR167653 treatment normalized GBM thickening and foot process widening. Immunofluorescence study showed that this treatment kept high intensity of nephrin and podocin in glomeruli. Furthermore, administration of FR167653 reduced the number of glomerular TUNEL-positive cells and p53 staining. *In vitro* study, overexpression of MKK3 in cultured human podocytes upregulated the phosphorylation of p38 MAPK and BAX mRNA expression, an apoptosis-related gene. Such induction was significantly inhibited by the administration of ANP. These results suggest that renoprotective properties of the endogenous natriuretic peptide/GC-A system may result from the local inhibition of aldosterone/p38 MAPK pathway and p53 signaling in podocytes.

GENOMICS OF HYPERTENSION IN DAILY MEDICAL PRACTICE

Tomohiro Katsuya
Katsuya Clinic, Amagasaki, Japan

Four and a half years have passed since I started my life as a physician of general practice. In Osaka University, I spent most of my time for the research of hypertension genomics, and the main aim of research was “identification of causative genes for hypertension”. Even though we carried out genome-wide association study (GWAS) using several different panels including huge number of subjects, it seems to be impossible to make a certain diagnosis of essential hypertension using only genetic information. In contrast, Prof. Lifton and his colleagues in Yale University have succeeded in identifying the causative genes of monogenic blood pressure abnormality, such as Liddle syndrome, etc. They also revealed that specific types of hypertension are caused by rare variants. In addition, recent investigations revealed that the epigenetic modification was also important in the pathogenesis of hypertension. For general physicians, genomic information concerning antihypertensive drug responsiveness, salt sensitivity and susceptibility of cardiovascular complications should be useful. In HOMED-BP-GENE study, we revealed that the responsiveness for the treatment was different among antihypertensive drugs. We also revealed high salt sensitivity of Japanese from our genetic association studies using classical candidate gene approach. In addition, beta-adrenergic receptor gene (*ADRB2*) polymorphism was revealed to be involved in obese hypertension and altered the responsiveness of life stile modification in a cohort study. *ADRB2* was also involved in the pathogenesis of salt sensitive hypertension *via* epigenetic modification of WNK family. Our recent investigation examining of the effect of ANRIL (antisense non-coding RNR in the INK4 locus) on multi-disease susceptibility suggested the future direction of genomic research in polygenic diseases. In this symposium, I would like to review my previous investigations and discuss the advantages and disadvantages of using genetic information in general clinical practice.

PRENATAL CORTISOL EXPOSURE AFFECTS THE BLOOD PRESSURE IN LATER LIFE

Fumiko Kawakami-Mori[1], Tatsuo Shimosawa[2], Daisuke Watanabe[1], Takashi Ando[1], Yasufumi Seki[1], Naohiro Yoshida[1], Satoshi Morimoto[1], Atsuhiro Ichihara[1]
[1]Hypertension and endocrinology, [2]Nephrology and encodrinology, Tokyo Women's Medical University, Tokyo, Japan

It is known that the prenatal stress is a risk factor for the metabolic disease and emotional disorders. This change is permanent and induced by epigenomic changes. The aim of this study is to investigate whether fetal stress would affect the blood pressure control in the central nervous system. We injected dexamethasone (200 mg/kg/d, Dex group) or saline (Control group) intraperitoneally to pregnant Sprague-Dawley rat (from F15 to F20). The body weight of Dex group (14.8±0.5 g) at the 7 post-natal day (P7) is lower than Control group (15.2±0.4 g, $P<0.05$), whereas it is much higher at the 12 weeks (Dex:552.5±3.9 g, S:393.8±9.4, $p< 0.001$). To investigate whether the blood pressure is elevated in the Dex group, we measured blood pressure using telemetry system or direct measurement *via* carotid artery with normal, 8 % salt (HS), and 0.1 % salt (LS) diet loading at the 12 weeks. Blood pressure is elevated after salt loading in Dex group (Dex_HS 159.5±3.4 mm Hg, Dex_NS group:142.8±1.9 mm Hg, $p<0.05$). Next we evaluated urine catecholamine, which is depressed by salt loading, and catecholamine decrease is weaker in the Dex group than Control group. Importantly suppression of catecholamine decrease is one of the causes of salt sensitivity. To examine whether DNA methylation status is affected by dex, we evaluated the methylation enzyme (DNMT1, DNMT3a, 3b) in hypothalamus and mRNA expressions of the DNMT3a and 3b were downregulated in the Dex group. Further, we injected demethylating agent, 5-Aza-2’-deoxycitydine intraventricularly to the male SD rats, measured the blood pressure loading HS and LS by telemetry system. Importantly DNA demethylation in the CNS induced salt sensitive hypertention. We hypothesized that renin-angiotensin system and ROS would be affected by metylation enzyme, because they are one of the key regulators of the blood pressure in the CNS. However, mRNA expressions of the AT1a, AT1b, NR3C1 and NR3C2 were not different

between Control and Dex group, nor NOX2 or NOX4, NADPH oxidase component, though the mRNA change by salt loading were different between groups. The DNA methylation state in the hypothalamus changed with the stress exposure in an embryo and a possibility that it had contributed to salt susceptibility high blood pressure was suggested.

EXAMINATION AS THE DIETARY NON-ALCHOHOLIC FATTY LIVER DISEASE MODEL OF SHRSP5/Dmcr

Kazuya Kitamori[1,2,3], Hiso Naito[2], Hazuki Tamada[1,2], Satoru Tsuchikura[3], Naomi Yasui[4], Katsumi Ikeda[3,4], Yukio Yamori[3,5], Tamie Nakajima[2,6]
[1]College of Human Life and Environment, Kinjo Gakuin University, Nagoya, Japan, [2]Graduate School of Medicine, Nagoya University, Nagoya, Japan, [3]Disease Model Cooperative Research Association, Kyoto, Japan, [4]School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Japan, [5]Institute for World Health Development, Mukogawa Women's University, Nishinomiya, Japan, [6]College of Life and Health Sciences, Chubu University, Kasugai, Japan

Non-alcoholic fatty liver disease includes a chronic progressive liver disorder from non-alcoholic fatty liver to non-alcoholic steatohepatitis with chronic inflammation and fibrosis of the liver. We developed SHRSP5/Dmcr for these models, which is a substrain developed from stroke-prone spontaneously hypertensive rat (SHRSP), the so-called "Arteriolipidosis-prone rats (ALR)" established in Kyoto University and Kinjo Gakuin University. When the rats were fed the high-fat and high-cholesterol containing (HFC) diet, their liver became white in color. Therefore, we examined the effects of the HFC diet intake period and the dose on liver damage of SHRSP5/Dmcr. Ten-week-old male SHRSP5/Dmcr rats were fed control (SP) or HFC diet for 2, 8 and 14 weeks. In another experiment, male SHRSP5/Dmcr rats were divided into 4 groups and fed 4 different diets, respectively: control diet (HFC: SP = 0:100), low-HFC diet (HFC: SP = 10 : 90), medium-HFC diet (HFC: SP = 50 : 50), and high-HFC diet (HFC: SP = 100 : 0) for 8 weeks. We examined the body and liver weight, blood pressure, several serum indices of liver function and hepatic histopathological changes. Body weights of rats fed HFC diet were lower compared with those of the control group. The blood pressure of HFC group was significantly lower than control at 14 weeks. The HFC diet time-dependently increased serum AST, ALT, γ-GTP levels. HFC diet increased microvesicular steatosis and inflammatory cell infiltrations at 2 weeks, Mallory-Denk body and bridging fibrosis in addition to macrovesicular steatosis, and inflammatory cell infiltrations at 8 weeks. At 14 weeks, honeycomb fibrosis was observed in HFC-fed rats. In another experiment, we found that the various liver damage indices mentioned above occurred in a dose-dependent fashion on the HFC diet. Interestingly, no liver damage occurred in rats fed the control and low-HFC diet. Thus, HFC diet time- and dose-dependently induces lipid accumulation, inflammation and severe fibrosis in the liver of SHRSP5/Dmcr. Their histological and biochemical changes are very similar to those of non-alchoholic fatty liver disease in human. Therefore, SHRSP5/Dmcr is thought to be a progressive model of the non-alchoholic fatty liver disease without the obesity.

RENOPROTECTIVE EFFECT OF DARBEPOETIN ALPHA IN DAHL RATS WITH HEART FAILURE

Naohiko Kobayashi[1], Yasuhiko Ueno[1], Yasuko Mamada[1], Keiko Fukuda[1], Hisato Hirata[2], Yoshifumi Machida[2], Noriko Suzuki[3], Fumie Yokotsuka[3], Kyoko Tabei[3], Machiko Sakata[3], Toshihiko Ishimitsu[1]
[1]Department of Cardiology and Nephrology, [2]Laboratory Animal Research Center, [3]Clinical Research Center, Dokkyo Medical University School of Medicine, Tochigi, Japan

Erythropoietin (EPO) improves cardiorenal function and induces neovascularization in chronic heart failure (CHF), although the exact mechanism has not been elucidated. We evaluate the renoprotective effects of chronic treatment with darbepoetin α (DPO) on renal dysfunction, podocyte injury, inflammation, oxidative stress, epithelial-mesenchymal transition (EMT), senescent gene, and endothelial progenitor cells (EPC) in the renal cortex of Dahl salt-sensitive hypertensive (DS) rats with CHF. DS and Dahl salt-resistant rats were fed a high-salt diet at 6 weeks of age. DS rats were treated with vehicle and DPO (1 μg/week) from the age of 11 to 18 weeks. Decreased percent fractional shortening in failing rats was significantly ameliorated by DPO. Vehicle-treated DS rats developed proteinuria, renal dysfunction, glomerulosclerosis, and interstitial fibrosis, which were ameliorated by DPO without changing blood pressure. Decreased expression of nephrin and podocin and increased desmin-positive area in failing rats were restored by DPO. Upregulation of NAD(P)H oxidase $p22^{phox}$, $p47^{phox}$, and $gp91^{phox}$, EMT marker such as α-SMA, TGF-β1, vimentin, and fibronectin, and aging signaling p53 expression in DS rats was significantly suppressed by DPO. DPO administration resulted in significant inhibition in TNF-α, MCP-1 and RANTES expression, and upregulation in klotho, sirt1 expression and CD34/Flk1-positive cells by FACS. We concluded that long-term DPO therapy may improve renal dysfunction, glomerulosclerosis, podocyte injury, and inflammation associated with oxidative stress, EMT, senescent gene and EPC in failing heart of DS rats. Thus, DPO may be a potential therapeutic strategy in CHF.

RENOPROTECTIVE EFFECT OF VASOPRESSIN V_2 RECEPTOR ANTAGONIST TOLVAPTAN IN DAHL RATS WITH HEART FAILURE

Naohiko Kobayashi[1], Yasuhiko Ueno[1], Yasuko Mamada[1], Keiko Fukuda[1], Hisato Hirata[2], Yoshifumi Machida[2], Noriko Suzuki[3], Fumie Yokotsuka[3], Kyoko Tabei[3], Machiko Sakata[3], Toshihiko Ishimitsu[1]
[1]Department of Cardiology and Nephrology, [2]Laboratory Animal Research Center, [3]Clinical Research Center, Dokkyo Medical University School of Medicine, Tochigi, Japan

Tolvaptan is the highly selective and orally effective arginine vasopressin V_2 receptor antagonists, and is potentially useful for treatment of heart failure (HF) patients. However, the renoprotective effect of long-term tolvaptan therapy and its underlying mechanisms remain unknown. We evaluate the effects of chronic treatment with tolvaptan on renal dysfunction, podocyte injury, inflammation, and oxidative stress, Rho-kinase, epithelial-mesenchymal transition (EMT), and extracelluar signal-regulated protein kinase (ERK1/2) pathway in the renal cortex of Dahl salt-sensitive hypertensive (DS) rats with end-stage severe HF. DS and Dahl salt-resistant rats were fed a high-salt diet at 6 weeks of age. DS rats were treated with vehicle and tolvaptan (0.05 % concentration in diet) from the age of 11 to 18 weeks. Vehicle-treated DS rats developed proteinuria, renal dysfunction, glomerulosclerosis, and interstitial fibrosis, which were ameliorated by tolvaptan without changing blood pressure. Decreased expression of nephrin and podocin and increased desmin-positive area in failing rats were restored by tolvaptan. Upregulation of NAD(P)H oxidase $p22^{phox}$, $p47^{phox}$, and $gp91^{phox}$, EMT marker such as transforming growth factor-β1, vimentin, and fibronectin expression, and Rho-kinase and ERK1/2 phosphorylation in DS rats was significantly suppressed by tolvaptan. Tolvaptan administration resulted in significant inhibition in tumor necrosis factor-α and monocyte chemoattractant protein-1 expression, and nuclear factor-κB phosphorylation. We concluded that long-term tolvaptan therapy may improve renal dysfunction, glomerulosclerosis, podocyte injury, and inflammation associated with oxidative stress, EMT, ERK, and Rho-kinase pathway in failing heart of DS rats. Thus, tolvaptan may be a therapeutic strategy for end-stage severe HF.

RENOPROTECTIVE MECHANISMS OF TELMISARTAN ON RENAL INJURY AND INFLAMMATION IN SHRSP.Z-LEPR(FA)/IZMDMCR RATS

Naohiko Kobayashi[1], Yasuhiko Ueno[1], Yasuko Mamada[1], Keiko Fukuda[1], Hisato Hirata[2], Yoshifumi Machida[2], Noriko Suzuki[3], Fumie Yokotsuka[3], Kyoko Tabei[3], Machiko Sakata[3], Toshihiko Ishimitsu[1]
[1]Department of Cardiology and Nephrology, [2]Laboratory Animal Research Center, [3]Clinical Research Center, Dokkyo Medical University School of Medicine, Tochigi, Japan

SHRSP.Z-$Lepr^{fa}$/IzmDmcr (SHRSP fatty) rats create a new animal model of metabolic syndrome. However, the renoprotective effect of telmisartan therapy and its underlying mechanisms in SHRSP fatty rats remain unknown. We evaluate the effects of long-term telmisartan

therapy on renal dysfunction, podocyte injury, inflammation, and transforming growth factor-β1 (TGF-β1)/Smad, epithelial-mesenchymal transition (EMT), mitogen-activated protein kinase (MAPK), Rho-kinase, and cell-cycle progression pathway in the renal cortex of SHRSP fatty rats. Seven-week-old male SHRSP fatty rats were treated with vehicle, telmisartan, and hydralazine for 8 weeks. Age-matched male Wistar-Kyoto/Izumo rats served as a control group. Vehicle-treated SHRSP fatty rats developed proteinuria and renal dysfunction, which in the telmisartan group was less than the vehicle and hydralazine group without changing blood pressure. Glomerulosclerosis and interstitial fibrosis were impaired in SHRSP fatty rats, and these renal damage in the telmisartan group was less than the vehicle and hydralazine group. Decreased expression of nephrin and podocin and increased desmin-positive area in SHRSP fatty rats were restored by telmisartan but not hydralazine. TGF-β1/Smad, EMT marker, MAPK, Rho-kinase, and cell-cycle progression pathways were upregulated in SHRSP fatty rats, and these increased proteins in the telmisartan group were less than the vehicle and hydralazine group. Telmisartan administration resulted in significant suppression in tumor necrosis factor-α expression and nuclear factor-κB phosphorylation. Long-term telmisartan therapy may improve renal dysfunction, glomerulosclerosis, podocyte injury, and inflammation associated with EMT, TGF-β/Smad, MAPK, Rho-kinase pathway in SHRSP fatty rats. Thus, telmisartan may have significant therapeutic potential for metabolic syndrome.

RENAL DAMAGE AFTER MYOCARDIAL INFARCTION IS PREVENTED BY LINAGLIPTIN, A DIPEPTIDYL PEPTIDASE INHIBITOR, FOR CARDIO-RENAL PROTECTION

Naohiko Kobayashi[1], Yasuhiko Ueno[1], Yasuko Mamada[1], Keiko Fukuda[1], Hisato Hirata[2], Yoshifumi Machida[2], Noriko Suzuki[3], Fumie Yokotsuka[3], Kyoko Tabei[3], Machiko Sakata[3], Toshihiko Ishimitsu[1]
[1]Department of Cardiology and Nephrology, [2]Laboratory Animal Research Center, [3]Clinical Research Center, Dokkyo Medical University School of Medicine, Tochigi, Japan

Myocardial infarction (MI) aggravates preexistent mild renal damage that is elicited by unilateral nephrectomy in rats. However, cardio-renal protection after MI is unclear. We evaluate whether linagliptin, a dipeptidyl peptidase inhibitor, improves cardiovascular remodeling and renal damage after MI in unilateral nephrectomized (UNX) Wistar rats. MI was induced after UNX by ligation of the left coronary artery (UMI). Vehicle and linagliptin were administration in UMI rats for 12 weeks. Impaired glomerulosclerosis and interstitial fibrosis in UMI rats were ameliorated by linagliptin. Podocyte injury such as decreased expression of nephrin and podocin and increased desmin-positive area in the renal cortex of UMI rats were restored by linagliptin. Upregulation of tumor necrosis factor-alpha expression and nuclear factor-kappaB phosphorylation and CD68-positive area in the renal cortex were suppressed by linagliptin. Increased cell-cycle progression marker such as cyclin D1/CDK4 and cyclin E/CDK2 expression in the renal cortex was blocked by linagliptin. Decreased %FS and increased fibrosis area in the LV was improved by linagliptin. UMI rats in the renal cortex and LV were characterized by increased NAD(P)H oxidase $p22^{phox}$, $p47^{phox}$, $gp91^{phox}$, and decreased eNOS phosphorylation, and linagliptin improved these indices. These findings suggest that cardio-renoprotective effect of linagliptin in cardio-renal damage after MI may play an important role in UMI rats. Thus, linagliptin may be a therapeutic strategy in the kidney and heart after MI.

RXR AGONIST HX630 MAY BE A NOVEL CANDIDATE FOR THE THERAPEUTICS AGAINST CUSHING'S DISEASE

Naotaka Kogure[1], Akiko Saito-Hakoda[1], Akira Uruno[2], Kyoko Shimizu[1], Kaori Sugawara[1], Yuko Iki[1], Tatsuaki Yoshikawa[3], Ken Matsuda[4], Masataka Kudo[4], Hiroyuki Kagechika[5], Yasumasa Iwasaki[6], Sadayoshi Ito[4], Akira Sugawara[1]
[1]Department of Molecular Endocrinology, [2]Department of Medical Biochemistory, [3]Department of Pharmacology, [4]Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan, [5]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan, [6]Health Service Center, Kochi University, Kochi, Japan

Cushing's disease is a disorder caused by excessive adrenocorticotropic hormone (ACTH) secretion from corticotroph adenomas in the anterior pituitary gland. So far, effective medications against Cushing's disease have not been established yet. Since nuclear retinoid X receptor (RXR) has been shown to be expressed in human pituitary corticotroph adenomas, we focused on the effects of RXR agonists on proopiomelanocortin (POMC) gene expression, mRNA expression, ACTH secretion, cell proliferation, and cell apoptosis using mouse pituitary corticotroph AtT20 cells. We incubated AtT20 cells with various concentrations of RXR agonists HX630, PA024, and CD3254, and analyzed POMC promoter activity by luciferase assay, POMC mRNA expression by real-time PCR, ACTH secretion by ELISA, cell proliferation by Cell Counting Kit-8, and cell apoptosis by Homogeneous Caspases Assay. HX630 and PA024, but not CD3254, inhibited AtT20 cell proliferation and induced AtT20 cell apoptosis. On the other hand, HX630, but not PA024 and CD3254, inhibited POMC gene transcription, POMC mRNA expression, and ACTH secretion. RXR agonist HX630 may therefore be a novel candidate for the therapeutics against Cushing's disease.

THERAPEUTIC ANGIOGENIC POTENTIAL OF DEDIFFERENTIATED FAT CELLS TO TREAT ISCHEMIC CARDIOVASCULAR DISEASES

Taro Matsumoto, Tomohiko Kazama, Kazuhiro Hagikura, Syogo Komiyama, Koichiro Kano, Noboru Fukuda
Department of Functional Morphology, Division of Cell Regeneration and Transplantation, Nihon University School of Medicine, Tokyo, Japan

Mature adipocytes are the most abundant cell type in adipose tissue. Using an *in vitro* dedifferentiation strategy, referred to as ceiling culture method, we established dedifferentiated fat (DFAT) cells from mature adipocytes. DFAT cells were capable of differentiating along multiple lineages and had significant expansion capability as similar as mesenchymal stem cells (MSCs). In the present study, we examined the potential impact of DFAT cells on angiogenesis and therapeutic angiogenesis. Mouse DFAT cells were cocultured with mouse vascular endothelial cells (GFP-MS1) for 7 days and immunostained for the pericyte markers α-smooth muscle actin (ASMA) and NG2. Nude mice were subcutaneously implanted with Matrigel plugs mixed with GFP-labeled DFAT cells for 2 weeks. Subsequently, gels were removed and processed for immunohistochemistry to detect GFP^+ vascular cells. Human DFAT cells, adipose-derived stem cells (ASCs), and bone marrow MSCs were cultured with 0.5 %FBS in DMEM for 3 days and conditioned media were taken and analyzed for cytokines using a cytokine array and ELISA. Twenty-four severe combined immunodeficiency (SCID) mice underwent right femoral artery dissection. Subsequently, DFAT cells (DFAT group, n =8) or peripheral mononuclear cells (PB-MNC group, n =8), or saline (Control group, n=8) were injected into ischemic muscle tissue. Blood flow was measured every week by a laser doppler blood flow meter. At 4 week after transplantation, ischemic limbs were processed for immunohistochemistry and number of isolectin B4 $(IB4)^+$ capillaries and $ASMA^+IB4^+$ capillaries were counted. In coculture conditions with mouse DFAT cells and mouse vascular endothelial cells (MS1), DFAT cells frequently differentiated into α-smooth muscle actin $(ASMA)^+$ $NG2^+$ pericyte-like cells. In Matrigel plug assay, transplanted DFAT cells differentiated $ASMA^+$ vascular smooth muscle cells in nude mice. In cytokine analysis of conditioned media, DFAT cells secreted several angiogenic cytokines, such as VEGF and HGF. The expression profiles of DFAT cells were very similar to those of ASCs and bone marrow MSCs. In a mouse ischemic hindlimb model, Blood flow was significantly increased in DFAT group compare to that in PB-MNC group or Control group from 2 to 4 weeks after transplantation ($p<0.05$). Density o $IB4^+$ capillaries and $ASMA^+IB^+$ capillaries in the ischemic muscle tissue was significantly increased in DFAT group compared to that in PB-MNC group or Control group ($p<0.05$). In conclusion, these data demonstrated that DFAT cells have the ability to differentiated to vascular cells *in vitro* and *in vivo*, and secrete several angiogenic factors. In addition, transplantation of DFAT cells led to neovascularization and improve blood flow in mice with ischemic hind limbs. We propose that DFAT cells represent a promising candidate cell source for angiogenic cell therapy in patients with ischemic cardiovascular diseases.

DIABETES PATIENTS WITH A HYPERTENSIVE RESPONSE TO EXERCISE HAVE IMPAIRED LEFT VENTRICULAR DIASTOLIC FUNCTION

Keishi Moriwaki[1], Tadafumi Sugimoto[1], Masahide Horiguchi[1], Takeshi Takamura[1], Shigetoshi Sakabe[1], Tetsuya Seko[1], Katsuya Onishi[3], Mashio Nakamura[2], Atsunobu Kasai[1], Masaaki Ito[3]
[1]Department of Cardiology, Ise red cross hospital, Mie, Japan, [2]Department of Clinical Cardiovascular Research, Mie University Graduate School of Medicine, Tsu, Japan, [3]Department of Cardiology and Nephrology, Mie University Graduate School of Medicine, Tsu, Japan

Elevated systolic load during exercise prolongs left ventricular (LV) relaxation, compromises filling, and raises end-diastolic pressure, leading to reduced exercise tolerance. We hypothesize that diabetes patients with a marked hypertensive response to exercise (HRE) have impaired LV diastolic function leading to exercise intolerance, even in the absence of resting hypertension. We recruited 238 subjects with type 2 diabetes, a preserved ejection fraction, a negative stress test, and a salt restriction diet (age 56±12 years, 60 % male, body mass index 24±5 kg/m^2, HbA1c 10.6±1.9 %). HRE (systolic blood pressure >160 mm Hg) was evaluated at the end of a 3-min exercise test using the Bruce protocol. Patients were categorized into three groups: a group without HRE and without resting hypertension (control group; n=102), a group with HRE but without resting hypertension (HRE group; n=73), and a group with resting hypertension (HTN group; n=63). Conventional Doppler and tissue Doppler imaging were performed at rest. Impaired diastolic function (E/E' >8) was found in 178 subjects (75 %) of which 15 (6 %) had diastolic dysfunction (E/E' >15). There were no significant differences in age, body mass index, HbA1c, LV end-diastolic volume, and LV ejection fraction, among the three groups. However, exercise duration was significantly shorter and E/E' was significantly higher in patients of the HRE and HTN groups compared to controls (control; 541±128 s and 9.0±2.6, HRE; 419±144 s and 10.3±3.0, HTN; 399±143 s and 11.2±3.9, respectively, $p<0.05$). After adjustment for age, gender, body mass index, HbA1c and resting systolic blood pressure, exercise systolic blood pressure after 3-min exercise tests was significantly associated with exercise duration ($\beta= -0.33$, $p<0.001$) in all subjects. In conclusion, irrespective of the presence of resting hypertension, diabetes patients with HRE had impaired LV diastolic function and exercise intolerance.

INFLUENCE OF LACTATION ON POSTPARTUM CARDIAC FUNCTION IN PREGNANCY-ASSOCIATED HYPERTENSIVE MICE

Kazuya Murata[1], Chiaki Saito[2], Junji Ishida[2], Juri Hamada[2],Fumihiro Sugiyama[3], Ken-ichi Yagami[3], and Akiyoshi Fukamizu[1,2]
[1]Life Science Center, Tsukuba Advanced Research Alliance, University of Tsukuba, Tsukuba, Japan, [2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan, [3]Laboratory Animal Resource Center, University of Tsukuba, Tsukuba, Japan

Pregnancy-induced hypertension (PIH) is a serious complication during pregnancy and recent epidemiological studies indicate the association between PIH and cardiac morbidity and mortality during the postpartum period. Although lactation is essential for the mammalian reproduction, the role of lactation in the heart with a prior of PIH remains unclear. In this study, we investigated postpartum changes in cardiac remodeling and function of pregnancy-associated hypertensive (PAH) mice with and without lactation. The systolic blood pressure was increased in PAH mice at day 19 of gestation (E19), and was reduced to normal levels in both lactating and non-lactating groups in the postpartum period. Histological analyses of heart revealed that hypertrophy and macrophage infiltration in PAH mice at E19 were improved in both lactating and non-lactating groups at 4 weeks postpartum (4W-PP), while marked fibrosis was remained. Increased mRNA expression of profibrotic genes and proinflammatory cytokines in PAH mice at E19 was significantly reduced in both lactating and non-lactating groups at 4W-PP. Echocardiographic analysis found no significant differences in fractional shortening between PAH mice and C57BL/6J mice at E19. On the other hand, at 4W-PP, non-lactating PAH mice showed normal fractional shortening, but lactating PAH mice exhibited significant decreases in cardiac contractility compared with non-lactating PAH mice. These results show that cardiac remodeling induced by hypertension during pregnancy is improved in the postpartum period except fibrosis, whereas lactation induces cardiac contractile dysfunction in mice with a history of pregnancy-associated hypertension.

CHANGES OF CHOLESTEROL AND BILE ACID KINETICS IN LIVER OF SHRSP5/DMCR FED A HIGH FAT-CHOLESTEROL DIET

Hisao Naito[1], Xiaofang Jia[1], Husna Yetti[1], Kazuya Kitamori[1,2], Hazuki Tamada[1,2], Yukio Yamori[3], Tamie Nakajima[1,4]
[1]Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]College of Human Life and Environment, Kinjo Gakuin University, Nagoya, Japan, [3]Institute for World Health Development, Mukogawa Women's University, Nishinomiya, Japan, [4]College of Life and Health Sciences, Chubu University, Kasugai, Japan

High fat-cholesterol (HFC) diet induced fibrotic steatohepatitis in liver of Stroke-Prone Spontaneously Hypertensive 5/Dmcr rat (SHRSP5/Dmcr). In this study, we aimed to clarify the molecular mechanism by analyzing the cholesterol and bile acid (BA) kinetics in the liver. Ten-week-old male SHRSP5/Dmcr rats were divided into two groups. One was fed SP (control) diet, and another was fed HFC diet. After feeding these diets for 2, 8 and 14 weeks, respectively, molecular markers involved in cholesterol and BA kinetics were investigated by measuring the protein and mRNA levels. Unlike the control diet, the HFC diet deposited much cholesterol in the livers, where 3-hydroxy-3-methylglutaryl CoA reductase, low-density lipoprotein (LDL) receptor and LDL receptor-related protein-1 were expectedly downregulated, especially at 8 and 14 weeks, suggesting that cholesterol synthesis and uptake may be inhibited in response to liver cholesterol accumulation. HFC diet increased BA synthesis by upregulating cholesterol 7 alpha-hydroxylase at 8 and 14 weeks. Unexpectedly, HFC diet did not inhibit feedback of BA synthesis by farnesoid X receptor. However, HFC diet suppressed canalicular BA excretion by decreasing the expression of bile salt export pump and multidrug resistance-associated protein-2 (MRP2) from 2 weeks because of the marked liver cholesterol accumulation. In contrast, HFC diet compensatorily increased basolateral BA excretion by increasing the expression of MRP3 at 8 and 14 weeks. Additionally, the HFC diet impaired BA detoxification by decreasing UDP-glucuronosyltransferase activity and sulfotransferase 2A1 expression at 8 and 14 weeks. The disturbed BA detoxification may correlate with suppressed pregnane X receptor and constitutive androstane receptor. The HFC diet increased the expressions of liver fibrosis makers such as transforming growth factor (TGF)-β1 (cytosol fraction) and α-smooth muscle actin (liver homogenate) at 8 and 14 weeks corresponding to liver fibrotic changes. HFC diet promoted BA synthesis, but decreased the bile duct excretion of BA and BA detoxification activity. As the result, the HFC diet may accumulate intrahepatic BA and exacerbate liver fibrosis.

BILE ACID ACCUMULATIONS IN THE LIVER OF RATS FED HIGH-FAT-CHOLESTEROL DIET AND THEIR RELATIONSHIPS WITH STEATOSIS, INFLAMMATION AND FIBROSIS INDICES

Tamie Nakajima[1,2], Xiaofang Jia[2], Hisao Naito[2], Akira Ishii[2], Husna Yetti[2], Kazuya Kitamori[3], Hazuki Tamada[2,3]
[1]College of Life and Health Sciences, Chubu University, Kasugai, Japan, [2]Nagoya University Graduate School of Medicine, Nagoya, Japan, [3]College of Human life and Environment, Kinjo Gakuin University, Nagoya, Japan

High-fat and -cholesterol (HFC) diet increased expressions of cytochrome P450 (CYP) 7A1 and CYP7B1 in the liver of stroke-prone spontaneously hypertensive 5/Dmcr (SHRSP5/Dmcr) rats, but decreased those of CYP27A1, CYP8B1 and bile salt export pump. These results suggested that HFC diet may dysregulate bile acids (BAs) in the liver. In this study, we measured 21 bile acids in the liver of rats fed HFC diet and investigated relationships between each bile acid and macrovesicular score, serum alanine aminotransferase (ALT) level and

fibrotic area. Male SHRSP5/Dmcr rats were fed with SP (control) or HFC diets for 2, 8 and 14 weeks, respectively. Hepatic 21 BA levels were determined by ultra-performance liquid chromatography-tandem mass spectrometry, using a published method. In the HFC group, the predominant BAs were CA, followed by deoxycholic acid (DCA) and CDCA species at 2 weeks. Cholic acid (CA) and chenodeoxycholic acid (CDCA) remained major species thereafter, but CA and DCA species dramatically decreased at 8 and/or 14 weeks; CDCA tended to increase after 2 weeks. As a result, CA/CDCA was 4 ~ 6 in control group, and about 1 in HFC group at 8 and 14 weeks. Unlike control, ursodeoxycholic acid (UDCA) species was a minor BA in the HFC group. Lithocholic acid (LCA), dehydrocholic acid α-/β-murichollic acid and hyodeoxycholic acid species were present in trace amounts in the liver of control and HFC groups. When glyco-BAs were compared with tauro-BAs, the HFC diet dramatically increased the former species but decreased the latter except for UDCA and LCA. CDCA species correlated positively, whereas total CA/total CDCA correlated negatively, with macrovesicular steatosis score, serum ALT and quantified fibrotic area. Total UDCA and tauro-CDCA also negatively correlated with these indices. Moreover, total glyco-BAs/total tauro-BAs positively correlated with each parameter of liver injury, whereas inverse associations were detected for total tauro-BAs. Hepatic BA accumulation may potentiate liver disease. CDCA plays a pivotal role in the pathogenesis of fibrotic steatohepatitis.

COMBINING IRBESARTAN AND TRICHLORMETHIAZIDE ELICITS STRONG BLOOD PRESSURE REDUCTION *VIA* INHIBITING SYMPATHETIC ACTIVITY WITHOUT ADVERSE EFFECTS ON METABOLISM IN HYPERTENSIVE RATS WITH METABOLIC SYNDROME

Masaaki Nishihara[1], Yoshitaka Hirooka[2]
[1]Department of Cardiovascular Medicine, [2]Department of Advanced Cardiovascular Regulation and Therapeutics, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

Sympathoexcitation plays a pivotal role in hypertension with metabolic syndrome. We have demonstrated that the oxidative stress in the rostral ventrolateral medulla (RVLM) increases sympathetic activity in hypertensive rats. The aim of the present study was to determine whether orally administered angiotensin II receptor blocker (irbesartan; IRB) and diuretics (trichlormethiazide; TCM) decreases blood pressure (BP) *via* inhibiting sympathetic activity through anti-oxidant effects in the RVLM of spontaneously hypertensive/NDmcr-cp rats (SHR-cp). We also investigated the effects of IRB/TCM therapy on the metabolic profile. IRB (25 mg/kg/day), TCM (1 mg/kg/day), IRB/TCM (25/1 mg/kg/day) or vehicle; VEH were orally administered for 28 days to SHR-cp. IRB/TCM treatment decreased BP to a greater extent than IRB monotherapy. Both urinary norepinephrine excretion and oxidative stress evaluated by TBARS levels in the RVLM were decreased in both IRB and IRB/TCM groups compared with VEH group, but not in TCM group. IRB/TCM treatment did not exert an adverse effect on the metabolic profile, such as fasting blood glucose, total cholesterol, LDL-cholesterol, and HDL-cholesterol, but it decreased the triglyceride levels of SHR-cp compared with VEH group. These findings suggest that IRB/TCM profoundly decreases BP by inhibiting sympathetic activity *via* anti-oxidant effect in the RVLM in SHR-cp.

'SALT MEMORY' FOR DEVELOPMENT OF HYPERTENSION IN HYPERTENSIVE ANIMALS

Hideyo Oguchi, Hiroyuki Sasamura, Kimiko Ishiguro, Kaori Hayashi, Mari Nakamura, Tatsuhiko Azegami, Hiroshi Itoh
Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan

We and others have shown that treatment of spontaneously hypertensive rats(SHR) or Dahl salt-sensitive rats with an renin-angiotensin-aldosterone(RAA) system inhibitor at the developmental phase of hypertension causes a sustained reduction of blood pressure. But the efficacy of salt intake in hypertension development is still unclear. The aim of this study was to examine the effects of temporary exposure to a high-salt diet on blood pressure and markers of end-organ damage in hypertensive rat models. (Experiment1) Dahl salt-sensitive rats were divided into 4 groups, and fed during an early stage of development (from age 6 to 14 weeks) with low-salt (0.12 % NaCl), normal-salt (0.8 % NaCl) , high-salt (7 % NaCl) or high-sodium/normal-chloride (12.7 % NaAA) diet. After these treatments, all groups were returned to a normal-salt diet. (Experiment 2) Male SHRs were divided into five groups, and fed from age 6 to 14 weeks with a low-salt, normal-salt, or high-salt diet. Other rats were given a 12.7 % NaAA or normal-sodium/high-chloride diet (11.6 % AACl). After these treatments, all groups were returned to a normal-salt diet. The effects on systolic blood pressure and urine protein excretion were examined regularly until age 24-28 weeks. (Experiment 1) Transient treatment with a high-salt diet caused an elevation in blood pressure not only during the treatment period, but also after returning to the normal-salt diet. 3 months after the treatment cessation, blood pressures were still elevated in the rats transiently exposed to a high-salt diet. Similarly, urine protein excretion was elevated in the high-salt rats at the end of the study. No such effect was seen in the NaAA group. At the age of 28 weeks, the high-salt group rats demonstrated renal arteriolar hypertrophy , and increases in systematic and renal RAA system activity. (Experiment 2) Transient treatment with a high-salt diet caused an elevation in blood pressure not only during the treatment period, but also after returning to the normal-salt diet. No such effect was seen in the NaAA or AACl groups. An increase in proteinuria and renal arteriolar hypertrophy was recognized in the high salt group, together with a marginal elevation in systematic and renal RAA system activity. In conclusion transient high dietary salt intake in the critical stage in the development of hypertension induces a sustained elevation of blood pressure, suggesting the presence of 'salt memory' in hypertensive animals. Both sodium and chloride ions were found to be essential for the development of this memory phenomenon. The mechanism of salt memory is suggested that high-salt diet intake in critical period of hypertension induces renal arteriolar remodeling through blood pressure rising, and elevates systematic and renal RAA activity.

VASCULAR PROTECTIVE EFFECTS OF ELASTIN PEPTIDE FROM BONITO BULBUS ARTERIOSUS IN SPONTANEOUSLY HYPERTENSIVE RATS

Kumiko Takemori[1], Ei Yamamoto[2], Hiroyuki Ito[2]
[1]Department of Food and Nutrition, Faculty of Agriculture, Kinki University, Japan, [2]Department of Biomedical Engineering, Faculty of Biology-Oriented Science and Technology, Kinki University, Japan

It is well known that the hypertrophy of vascular smooth muscle cells and degeneration or degradation of elastic fiber in the arterial wall is responsible for the development of hypertension and vascular injuries. In this study, we examined the effects of elastin peptide from bonito bulbus arteriosus on vascular walls in Spontaneously hypertensive rats (SHR/Izm). Male SHR were divided into two groups at 15 weeks of age. Elastin peptide (3 g/kg, in Funabashi SP) from bonito bulbus was administered for 5 weeks. Plasma proryl glysin (a degradation product of elastin peptide) levels were measured and endothelial changes were observed using scanning electron microscope (SEM). We further investigated the vascular function and expression of endothelial nitric oxide synthase (eNOS), intercellular adhesion molecule1 (ICAM-1) in endothelial cells and connective tissue related gene in the aorta by realtime PCR analysis. No significant differences were found in blood pressure, body weight, vascular function and connective tissue-related gene expression between the control and the elastin treated groups. Morphologically, the surface of the endothelium was smoother and endothelial changes such as blebs or crater-like structures were much less intense as compared to those in the control group. The expression of ICAM-1 was significantly lower in the elastin treated group than in the control, but not the expression of eNOS. These results indicate that PG (degradation product of elastin peptide) has protective effects against endothelial injuries through the inhibition of adhesion in endothelial cells and leukocytes. We plan to do more research to elucidate the mechanisms of such beneficial effects of elastin peptide, such as regarding elastin receptor, signal transduction and so on, using cultured endothelial cells and smooth muscle cells.

THE DIFFERENCE OF THE INFLUENCE OF AUTONOMIC NERVOUS ACTIVATION CAUSED BY REACTIVE HYPEREMIA ON TWO DIFFERENT ENDOTHELIAL FUNCTION TESTS

Masanobu Yoshida, Hirofumi Tomiyama, Mari Odaira, Kazuki Shiina, Akira Yamashina
Department of Cardiology, Tokyo Medical University, Tokyo, Japan

While reactive hyperemia is used for the assessment of endothelial function in clinical settings, this maneuver is stressful and may affect autonomic nervous system activity. Two different methods to assess endothelial function are available {i.e., conduit arterial endothelial function: flow-mediated vasodilatation (FMD), micro-vascular endothelial function: reactive hyperemia index assessed by both (left/right) finger-tips arterial tonometry (RHIendPAT)}, but influence of the changes of autonomic nervous activity related with reactive hyperemia on these two methods has not been clarified. The present study was conducted to examine the influence of the changes of autonomic nerve activitiy caused by reactive hyperemia on micro-vascular and conduit arterial endothelial function. In 108 hypertensive patients, FMD and RHIendPAT were measured simultaneously. The analysis of heart rate viability (HRV) {high frequency domain (HF), low frequency domain (LF) and their ratio (LF/HF)} were performed throughout this reactive hyperemia in these patients. By reactive hyperemia, LF was increased in 60 patinets (LFinc group) and was not increased in 48 patinets (LFdec group). RHIendPAT was lower in the former group (LFinc group) (2.2±0.5) than that in the latter group (LFdec group) (2.5±0.7) ($p<0.05$). However, no difference was found in FMD between these groups (3.4±2.0 % vs 3.9±2.5 %, $p=0.28$). Multivariate linear regression analysis demonstrated that the change of LF by reactive hyperemia was a significant determinant for RHIendPAT ($\beta= -0.23$, $p<0.05$), but not for FMD($\beta= -0.10$, $p=0.33$), independent of conventional risk factors for cardiovascular disease. The influence of the changes of autonomic nerve activity by reactive hyperemia might be significant on RHIendPAT, but not on FMD, even with the adjustment of simultaneous both sides (left and right fingers) measurements of finger-tip tonometry.