Short-term rapid atrial pacing alters the gene expression profile of rat liver: Cardiohepatic interaction in atrial fibrillation

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BACKGROUND Atrial fibrillation (AF) activates the coagulation system, leading to hypercoagulation of the blood. The liver is a major source of prothrombotic molecules.

OBJECTIVE This study aimed to clarify whether cardiohepatic interactions are involved in AF-related hypercoagulation.

METHODS We compared gene expression profiles of human liver tissue between patients with AF and sinus rhythm. An AF model was created by rapid atrial pacing (RAP) at a frequency of 1200 beats/min in anesthetized 10-week-old Sprague-Dawley rats. Livers, atria, and peripheral blood cells were collected and analyzed after 12 hours of RAP.

RESULTS DNA microarray analysis revealed marked changes in the gene expression profile of human liver of patients with AF. The extrinsic prothrombin activation pathway showed the most prominent change in 354 BioCarta pathways. Twelve hours of RAP also markedly altered the gene expression profile of rat liver. RAP markedly augmented the hepatic messenger RNA expression of fibrinogen chains, prothrombin, coagulation factor X, and antithrombin III. The augmented fibrinogen production by RAP was accompanied by increased of interleukin 6 (IL-6) messenger RNA expression in peripheral blood cells, enhanced monocyte chemoattractant protein-1 expression in the liver, infiltrated cluster of differentiation 11b–positive mononuclear cells in the liver, and enhanced signal transducer and activator of transcription 3 (STAT3) phosphorylation in the nuclei of hepatocytes. STAT3 phosphorylation and increased fibrinogen and coagulation factor X production by RAP were suppressed by pretreatment with IL-6 neutralizing antibody.

CONCLUSION Rapid atrial excitation mimicking paroxysmal AF remotely altered the hepatic gene expression of prothrombotic molecules. Increased fibrinogen expression in the liver by RAP was mediated by activation of the IL-6/STAT3 signaling pathway in the peripheral blood and liver.

KEYWORDS Atrial fibrillation; Fibrinogen; IL-6/STAT3 signaling pathway; Liver; Monocyte/macrophage

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Introduction

Atrial fibrillation (AF), the most common type of sustained arrhythmia in adults, is associated with a 5-fold increase in ischemic stroke incidences.[1,2] Most strokes in patients with AF are believed to be cardioembolic, caused by the embolism of left atrial thrombi.[3] As shown by the well-known Virchow triad, thrombus formation in the left atrium (LA) can result from decreased blood flow, increased endocardial dysfunction in the LA, and enhanced blood coagulability.[4,5] Spontaneous echo contrast formation in the LA is promoted by decreased blood flow velocity and is strongly associated with left atrial appendage thrombus and cardioembolic events.[6] Previous studies have also determined that AF-associated endocardial dysfunction is characterized by an increase in von Willebrand factor, nitric oxide, and plasminogen activator inhibitor-1 expression and a decrease in nitric oxide synthase expression in the LA.[7,8] Even short-term rapid atrial pacing (RAP) for 8 hours induces a marked decrease in tissue factor pathway inhibitor and thrombomodulin expression in the atrial endocardium of rats.[9] However, the mechanism of AF-induced hypercoagulation of the blood, connected with the third element of Virchow triad, is poorly understood. Because the liver is an essential organ synthesizing many coagulation factors and other prothrombic molecules, we hypothesized that a cardiohepatic interaction is involved in AF. Herein, we examined whether short-term rapid atrial excitation affects gene expression remotely in the liver.

Methods

Detailed methods can be found in the Online Supplemental Material.

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RAP model

This study was conducted using Sprague-Dawley rats (8–12 weeks of age) in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health. It was approved by the Institutional Animal Use and Care Committee of Kanazawa University. An atrial tachycardia rat model was prepared as previously reported.[9,10] Right atrial stimulation at a frequency of 1200 beats/min was performed with 2-ms rectangular pulses using a programmable stimulator. Sham surgery rats underwent an identical procedure without electrical stimulation. The details are given in the Online Supplemental Methods.

Human liver tissue

We screened 465 consecutive patients with nonalcoholic steatohepatitis who underwent liver biopsy from 2003 to 2013 and identified 3 patients with AF without structural heart disease. Three patients who were in sinus rhythm and matched for age, sex, and hepatic histopathological stage (Brunt's pathologic criteria) served as a control group (Online Supplemental Table 1). There was no statistical difference between the 2 groups in left atrial diameter. Informed consent was obtained from all patients before liver biopsy. The experimental protocol was approved by the Ethics Committee for Human Genome/Gene Analysis Research at Kanazawa University (approval no. 2014-002), and the study was conducted in accordance with the Declaration of Helsinki.

Microarray experiments, quantitative real-time polymerase chain reactions, Western blot analyses, and histological analyses

We performed microarray experiments using RNA from liver tissues of humans and rats. Quantitative real-time polymerase chain reaction was performed using RNA from the liver, LA, spleen tissues, and peripheral blood cells of rats. Western blot, immunohistochemical staining, and immunofluorescent staining were performed using liver tissues of rats. The detailed methods are presented in the Online Supplemental Methods.

Administration of interleukin 6 neutralizing antibody

Interleukin 6 (IL-6) neutralizing antibody (5 μg/400 kg of body weight) was intraperitoneally administered 1 day before and immediately after RAP onset.

Statistical analysis

Continuous variables are presented as mean ± SEM for each group unless stated otherwise and were analyzed by analysis of variance with Bonferroni post hoc testing for multiple comparisons and unpaired t test for 2-group comparisons. Categorical variables were compared using the χ^2 test. Significance was set at $P < .05$.

Results

Gene expression profiles of human liver in AF

DNA microarray revealed marked changes in the gene expression profile of the human liver of patients with AF. Of 54,675 human genes in the array, 4322 filtered genes demonstrated clear clusters for AF or control (Online Supplemental Figure 1). Table 1 displays significantly overrepresented BioCarta pathways in the liver of patients with AF. The expression of genes related to coagulation, immunity, apoptosis, and hypoxia was significantly altered in AF. Notably, the extrinsic prothrombin activation pathway, including fibrinogen, showed the most prominent change in 354 BioCarta Pathways (Table 1).

Characteristics of experimental rats

At the time of euthanasia, an electrocardiogram confirmed atrial-paced rhythm in each experimental animal. Arterial blood pressure was not different between 12-hour RAP and sham groups (Online Supplemental Figure 2A). Atrial pacing of 1200 beats/min brought the ventricular response from 2:1 to 3:1. Accordingly, the mean ventricular rates of the RAP model demonstrated a slightly higher trend but no statistical difference than did the sham model (Online Supplemental Figure 2B). In addition, there was no statistical difference between the groups in liver weight/body weight ratios, arterial blood gas parameters, or serum liver enzymes (Table 2).

Table 1 Significantly overrepresented BioCarta pathways in the liver of patients with AF

Gene and frequent pathway	LS permutation *P* value
Extrinsic prothrombin activation pathway	.00001
Overview of the telomerase protein component gene hTert transcriptional regulation	.00001
Complement pathway	.00016
p53 signaling pathway	.00020
Hypoxia and p53 in the cardiovascular system	.00027
CTCF: first multivalent nuclear factor	.00040
Estrogen-responsive protein Efp controls cell cycle and breast tumor growth	.00062
Acute myocardial infarction	.00068
Apoptotic signaling in response to DNA damage	.00088
Lectin-induced complement pathway	.00098
Downregulated MTA-3 in ER-negative breast tumors	.00173
ER-associated degradation pathway	.00222
IL4 signaling pathway	.00254
Basic mechanism of action of PPARa, PPARb(d), and PPARg and effects on gene expression	.00342
Activation of cAMP-dependent protein kinase, PKA	.00364
Regulation of elF4e and p70 S6 kinase	.00373
Signaling pathway from G-protein families	.00407

hTert = human teromerase reverse transcriptase; p53 = tumor protein p53; CTCF = CCCTC-binding factor; Efp = elongation factor p; DNA = deoxyribonucleic acid; MTA-3 = matastasis-associated protein 3; ER = endoplastic reticulum; IL4 = interleukin-4; PPAR = peroxisome proliferator activated receptor; cAMP = cyclic adenosine monophosphate; PKA = protein kinase A; eIF4e = eukaryotic translation initiation factor 4E; p70-S6 = ribosomal protein S6.