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ID No.: P16117

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Be sure to enter the Fellow's ID number

Form 7／様式 7
外国人特別研究員作成／By Fellow

2018 / 12 / 21
(YYYY) (MM) (DD)

JSPS Fellow's
Signature (Handwritten only) : KOID Suang Suang

Research Report (by Fellow)
(Cover Page)

I hereby submit the research report of my fellowship.

1. Name (Print) : KOID Suang Suang
2. Nationality : Malaysian
3. Host Institution : International University of Health and Welfare
4. Host Researcher : Professor SHIMOSAWA Tatsuo
5. Title of Research in Japan :
~~Inflammatory cell populations in a mouse model of cardiorenal syndrome~~
Role of kinin and angiotensin upon inflamatory responses in cardioreal syndrome
6. Fellowship Tenure : From 2016 / 4 / 1 (YYYY) (MM) (DD) To 2018 / 11 / 30 (YYYY) (MM) (DD)

***Notes for writing the Research Report**
***Type this form except the date and the signature.**
Please prepare your Research Report in English or Japanese within three to ten pages including this page. The contents should include:

7. Background of Research

Cardiorenal syndrome (CRS) is a condition in which renal dysfunction coexists with myocardial infarction and heart failure [Bongartz et al. 2005, Eur Heart J 26:11]. CRS results in accelerated failure of the heart and kidney via similar pathophysiological mechanisms. Renal failure is one of the strongest independent risk factors and predictors of mortality in patients with HF [Forman et al. 2004, J Am Coll Cardiol 43:61], although the pathogenesis of CRS remains poorly understood. A complex combination of hemodynamic, neurohormonal, immunological and biochemical feedback pathways contribute to CRS [Liu et al. 2012, Clin Exp Pharmacol Physiol 39:692].

Preliminary evidence suggests that inflammatory cells such as monocytes and macrophages may contribute to the pathogenesis of CRS. We (Shimosawa et al.) have demonstrated that the leukocyte-specific integrin Mac-1 links inflammation and thrombosis following glomerular injury [Hirahashi et al. 2009, Circulation 120:1255]. A more recent study suggested that monocyte activation by uremic toxins may be responsible for many aspects of the pathology of CRS [Armstrong et al. 2013, J Leukoc Biol 93:821]. However, animal models of CRS remain limited and the roles of specific inflammatory cell subsets in CRS remain poorly understood.

The main aims of this research were to establish a mouse model of CRS and to determine how inflammatory cell populations change as CRS progresses. Supplementary aim was to investigate the effect of angiotensin receptor blockers and angiotensin converting enzyme inhibitors on the change in inflammatory cell populations.

8. Research methodology

Establishment of a mouse model of mild kidney dysfunction

To be able to understand the role of inflammatory cells in the manifestation of CRS, we needed a model of mild but chronic kidney dysfunction to mimic the condition of asymptomatic patients, which would then be useful for development of therapeutic strategies at early stages of the condition. Three-week-old rats that were subjected to left uninephrectomy and high salt diet (UNIX/HS; 8% NaCl) developed severe kidney dysfunction. However, because C57BL/6J mice are relatively resistant to kidney damage, we decided to use a similar protocol on C57BL/6J mice to achieve a mouse model of mild kidney dysfunction.

In brief, three-week-old C57BL/6J mice were subjected to sham uninephrectomy and normal salt diet (SHAM/NS; 0.3% NaCl) or left uninephrectomy and high salt diet (UNIX/HS; 8% NaCl). After 4, 6 or 8 weeks of treatment, urine was collected for salt and protein analyses. The heart and right kidney were collected and histochemical staining was performed on sections of the heart and kidney.

Establishment of cardiorenal syndrome model (with acute myocardial infarction)

Based on the data from the mouse model of mild kidney dysfunction, which showed more stable kidney dysfunction 8 weeks after treatment, we chose the 8-week timepoint for the onset of acute myocardial infarction (MI).

In brief, three-week-old C57BL/6J mice were subjected to SHAM/NS or left UNIX/HS. After 8 weeks of treatment, the mice underwent acute MI. The mice were anesthetized and mechanically ventilated before thoracotomy and ligation of the left anterior descending (LAD) coronary artery. The mice underwent 30 minutes of ischemia and 2 hours of reperfusion. The LAD coronary artery was religated and blood was collected. The tissues (heart, kidney, spleen, bone marrow) were then collected for analyses of inflammatory cell distribution by flow cytometry.

Establishment of cardiorenal syndrome model (with chronic myocardial infarction)

In this model, we also chose the 6-week timepoint for the onset of chronic myocardial infarction (CMI). In brief, three-week-old C57BL/6J mice were subjected to SHAM/NS or left UNIX/HS. After 6 weeks of treatment, the mice underwent CMI. The mice were anesthetized and mechanically ventilated before thoracotomy and permanent ligation of the LAD coronary artery. 8 weeks post-surgery, blood and tissues (heart, kidney, spleen, bone marrow) were collected for analyses of inflammatory cell distribution by flow cytometry, and histochemical staining was performed on sections of the heart and kidney.

9. Results/impacts

Note: As much as possible, describe the contents and results of your research in a manner that is easily understandable to a non-specialist in your field. Provide a concrete description if (1) papers related to your work have been published in major academic journals, (2) particularly outstanding research results were achieved, or (3) patent applications have been made or other tangible outcomes achieved through the research.

We characterized a mouse model of kidney failure by performing uninephrectomy on mice at exactly three weeks of age, at the time of weaning, and then by maintaining mice on a high salt diet (8% NaCl). Previous studies in our laboratory have shown that performing uninephrectomy at later times points is insufficient to induce kidney dysfunction. We have also adapted and optimized our rat myocardial infarction protocols to work in mice, particularly microsurgery procedures, anesthetic and ventilation settings.

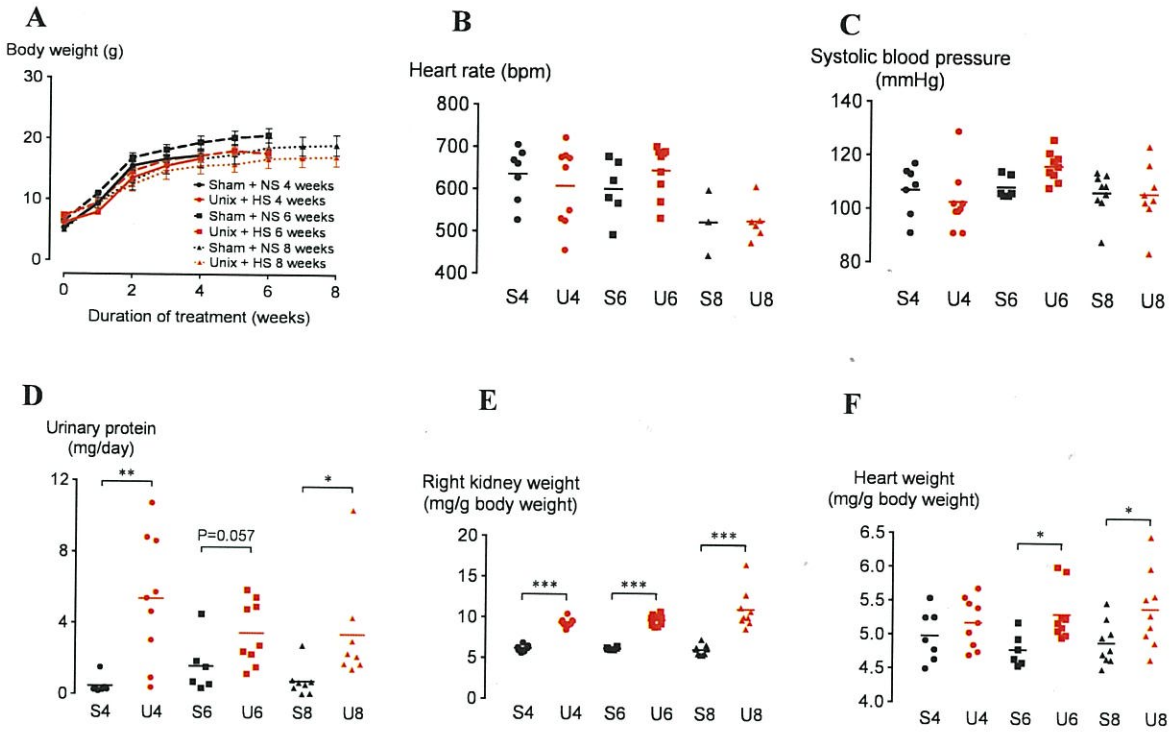

Figure 1. Physiological characteristics of SHAM/NS and UNIX/HS mice at 4, 6 and 8 weeks after uninephrectomy surgery.

Body weight (A), heart rate (B) and systolic blood pressure (C) did not differ when comparing SHAM/NS and UNIX/HS at the different time points. Urinary protein levels (D) were significantly higher in UNIX/HS mice from 4 weeks after uninephrectomy and high salt treatment, suggesting the development of kidney dysfunction. Right kidney weight/body weight ratio (E) is also significantly higher in the UNIX/HS group. This effect is expected because the removal of the left kidney causes feedback hypertrophy of the right kidney. Interestingly, even though the mice have not had any insults to the heart, the heart weight/body weight ratio (F) is higher in the UNIX/HS group as compared to the SHAM/NS group. This suggests that there may be crosstalk between the kidney and the heart. S = SHAM/NS; U = UNIX/HS; Data shown as means ± SEM; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 (comparisons as indicated; one-way ANOVA).

We have since applied the myocardial infarction (MI) conditions for our mouse model of kidney failure to establish 2 different mice models of CRS. In the acute (AMI) setting, mice (with or without uninephrectomy and high salt diet) are subjected to 30 min of ischemia to the left anterior descending coronary artery followed by 120 min of reperfusion. Using flow cytometry, our results showed increases in inflammatory monocytes and neutrophils in the heart of mice subjected to acute myocardial infarction at 8 weeks post uninephrectomy. Only increases in neutrophils are seen in the kidney (data not shown). Analysis of acute myocardial

infarction experiments in uninephrectomized mice and sham mice in the presence of drug treatments are ongoing.

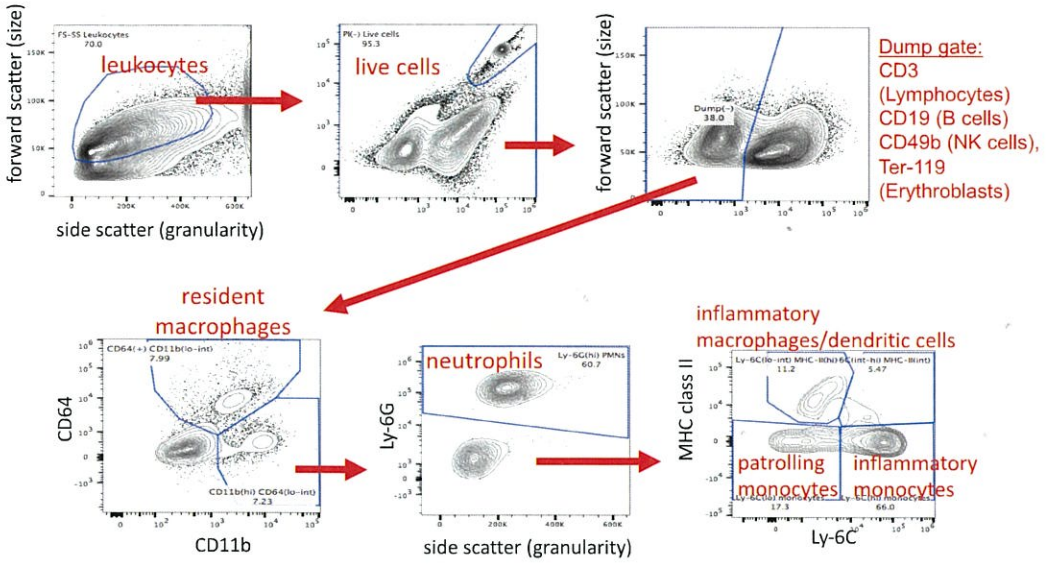

Figure 2. Gating strategy for identification of inflammatory cell populations in the heart and kidney.

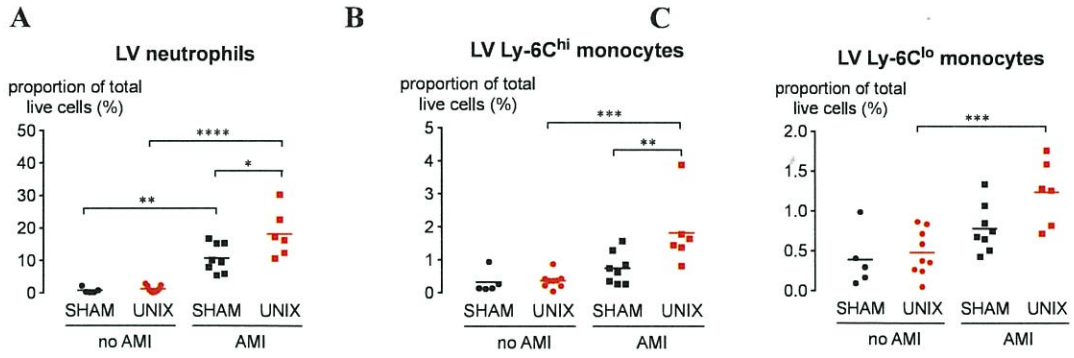

Figure 3. Neutrophils (A), Ly-6C^{hi} (B) and Ly-6C^{lo} (C) monocyte populations in the heart (left ventricle) of SHAM/NS and UNIX/HS mice subjected to sham surgery or AMI. (A) There is a significantly higher proportion of neutrophils with AMI (c.f. no AMI). Interestingly, comparing within the groups that underwent AMI, there is an even higher proportion of neutrophils in the heart of the UNIX/HS group (c.f SHAM/NS). (B) Again, there is a significantly higher proportion of Ly-6C^{hi} monocytes in the heart of the UNIX/HS group with AMI (c.f SHAM/NS with AMI). (C) A similar trend is seen with the Ly-6C^{lo} monocytes, suggesting that chronic kidney disease (even though mild) increases the inflammatory responses within the body. There are no changes in macrophage populations in the heart (data not shown). Data shown as means ± SEM; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 (comparisons as indicated; one-way ANOVA).

In chronic myocardial infarction (CMI), the left anterior descending coronary artery is permanently ligated and mice are maintained for 8 weeks prior to flow cytometry analysis. In the chronic setting however, monocyte and neutrophil populations were no different among the groups. Organ damage (fibrotic changes) analysis among the groups are also ongoing.

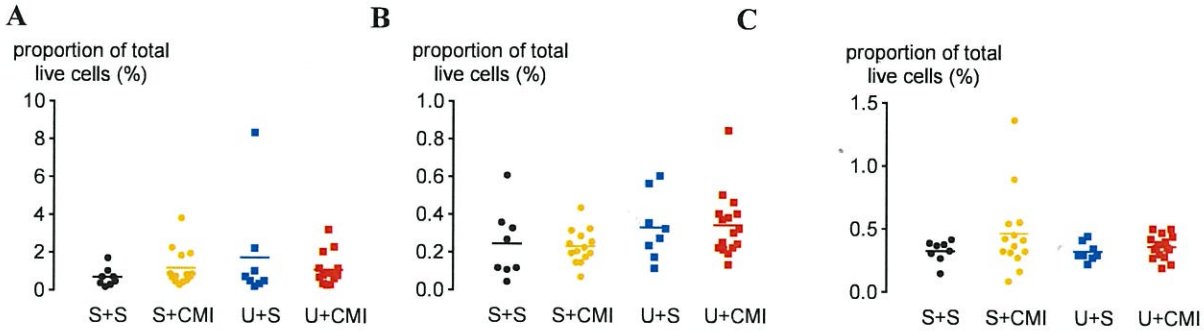

Figure 4. Neutrophils (A), Ly-6C^{hi} (B) and Ly-6C^{lo} (C) monocyte populations in the heart (left ventricle) of SHAM/NS and UNIX/HS mice subjected to sham surgery or CMI. The proportion of neutropil and monocyte populations were similar among the groups, suggesting the stabilization of the condition 8 weeks after the initial CMI insult.
S+S = SHAM/NS + SHAM CMI; S+CMI = SHAM/NS + CMI; U+S = UNIX/HS + SHAM CMI; U+CMI = UNIX/HS + CMI.

In summary,

- UNIX/HS resulted in kidney dysfunction and an increase in HW/BW ratio in C57BL/6J mice.
- AMI resulted in increased recruitment of Ly-6C^{hi} monocytes and neutrophils in the heart of UNIX/HS mice as compared to SHAM/NS mice, suggesting a role of these cells in the pathogenesis of CRS.
- Monocyte and neutrophil populations were no different among the groups, suggesting the stabilization of the condition 8 weeks after initial CMI insult.

Taken together, these results demonstrated progressive development of kidney dysfunction with associated heart pathology, consistent with the development of CRS. This model may aid the development of therapeutic strategies targeting inflammatory signaling pathways and modulating specific inflammatory cell populations in CRS.

Collaborative research during the period of the fellowship

- Examine the monocyte and macrophage distribution in Fucci mice bearing LLC subcutaneous tumors
- Examine the effect of a macrophage-targeted therapy on tumor growth and myeloid cell dynamics in the MMTV-PyVT tumor model

10. Research Presentations during the period of the fellowship (Name of the conference, title, place, date)

- 14th Vascular Pathology Research Seminar; Tokyo, Japan, 2018
  Presentation title: Immune cell populations in a mouse model of cardiorenal syndrome

- American Heart Association: Hypertension meeting; Chicago, USA, 2018
  Presentation title: Immune cell populations in a mouse model of cardiorenal syndrome

- 19th Takeda Science Foundation Symposium on Bioscience: Chronic Inflammation – Initiation, Progression and Resolution; Osaka, Japan, 2017
  Presentation title: Immune cell populations in a mouse model of cardiorenal syndrome

- International Society of Hypertension; Seoul, South Korea, 2016
  Presentation title: Cardioprotection by aliskiren, valsartan and their combination in rats with chronic myocardial infarction

- Japanese Society of Hypertension annual scientific meeting; Sendai, Japan, 2016
  Presentation title: Cardioprotection by aliskiren, valsartan and their combination in rats with chronic myocardial infarction

11. A list of paper published during or after the period of the fellowship, and the names of the journals in which they appeared (Please fill in the format below). Attach a copy of each article if available.

Author(s)	Title	Name of	Volume	Page	Date	Note
Koid SS Ziogas J Campbell DJ	Cardioprotection by aliskiren, valsartan and their combination in rats with chronic myocardial infarction.	Journal of Hypertension	34	e276	1 Sep 2016	
Reheman L Hirohama D **Koid SS** Wang C Mori F Yeerbolati A Liu B Yatomi Y Fujita T Shimosawa T	Mineralocorticoid Receptor is Responsible for Salt-Induced COX2 Expression in Renal Medulla	Journal of Hypertension	34	e424	1 Sep 2016	
Wang C Reheman L Ogura S **Koid SS** Mori F Shimosawa T	Low Dose LNAME Causes Salt Sensitive Hypertension via Activation of NCC	Hypertension	70	Suppl	1 Sep 2017	

Shand FHW **Koid SS** Ueha S Matsushima K	Monocyte maturation stage determines preferential recruitment to solid tumors in mice: Mo-p13-11	Cytokine	100	121	1 Dec 2017	
Koid SS Shand FHW Ueha S Matsushima K Shimosawa T	Inflammatory cell populations in a mouse model of cardiorenal syndrome	Journal of Hypertension	36	e45	1 Oct 2018	
Wang C Reheman L Ogura S **Koid SS** Mori F Shimosawa T	Low dose N-nitro-L-arginine Methyl Ester (L-NAME) causes salt sensitive hypertension via activation of NA^{+}-Cl^{-} cotransporter (NCC)	Journal of Hypertension	36	e4	1 Oct 2018	

12. Awards during the period of the fellowship (Name of the award, Institution, date etc.)

- Best poster presentation awards
  - 14th Vascular Pathology Research Seminar, 2018
  - International Society of Hypertension (ISH), 2016

- ISH Investigator Grant, International Society of Hypertension, 2016