# 腎不全マウスモデルにおける動脈硬化とスカベンジャーリセプター発現との関連

研究課題番号

09671184

平成9年～平成10年度科学研究費補助金（基盤研究C）

研究成果報告書



平成12年3月

研究代表者　安藤　稔

（東京女子医科大学医学部　助手）

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はしがき

腎不全特に透析患者における死亡原因疾患は心血管系性疾患でありその根本には、動脈硬化の進展が急速であるという事実が関与していると思われる。当該研究では、腎不全に特異的な動脈硬化進展機序が存在し、その一つとして腎不全では、酸化ＬＤＬを血管内皮下に取り込むｱｸｾｽとなる単球系細胞の発現するスカベンジャー受容体の発現亢進が起こりうるとの仮説を主としてヒトおよび腎不全ﾏｳｽを用いた実験により証明すべく意図された研究である。

平成12年3月

研究組織

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研究経費

| | |
|---|---|
| 平成9年度 | 1,500千円 |
| 平成10年度 | 500千円 |
| 計 | 2,000千円 |

研究発表

（1）学会誌

1． Ando M, Lundkvist I, Bergstrom J, Lindholm B: Enhanced scavenger receptor expression in monocyte-macrophages in dialysis patients. Kidney Int 49:773-780,1996

2. Ando M, Gafvels M, Bergstrom J, Lindholm B, Lundkvist I: Uremic serum enhances scavenger receptor expression and activity in the human monocytic cell line U937

3. Ando M, Lindholm B, Bergstrom J, Lundkvist I: Clinical relevance of elevated serum levels of M-CSF to secondary hyperparathyroidism and accelerated atherosclerosis in dialysis patients. Kidney Int (投稿予定)

4. Ando M, Sanaka T, Nihei H: Eicosapentanoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients. J Am Soc Nephrol 10:2177-2184,1999

5. Ando M,et al: A novel 18-amino acid deletion in apolipoprotein E associated with lipoprotein glomerulopathy. Kidney Int 56:1317-1323,1999

6. Ando M, et ai: New insights into the thrombopoietic status of hemodialysis patients through evaluation of megakaryocytopoiesis in the bone marrow and endogenous thrombopoietin levels. (Submitted to Blood)

（2）口頭発表

1.安藤　稔他：尿毒症における血清Ｍ－ＣＳＦ発現の亢進：その機序と動脈硬化との関連。日本内科学会（平成9年4月20-21日）

2.Ando M, et al: Clinical relevance of elevated serum M-CSF in dialysis patients. International Society of Nephrology Meeting, May 5-9, 1997

3.安藤　稔他：二次性上皮正体機能亢進症とＭ－ＣＳＦ上昇機序。日本透析医学会（平成9年7月3-5日）

4.安藤　稔：慢性腎不全における脂質代謝異常と動脈硬化：マクロファージスカベンジャー受容体の発現亢進との関連ー（平成10年6月15日）

5..安藤　稔：　慢性腎不全における高Ｍ－ＣＳＦ血症とスカベンジャーリセプター発現亢進と動脈硬化。ﾌﾘｰﾗｼﾞｶﾙ研究会（平成10年10月16日）

研究成果

Research I. Clinical significance and characterization of elevated serum M-CSF in dialysis patients with emphasis on atherosclerosis

Background: Macrophage scavenger receptor (SR) plays a key role in the initiation and/or progression of atherosclerosis. That could be also associated with the atheroiscrerosis in uremic (dialysis) patients. We have already demonstrated the scavenger receptors expressions are accerelated during the maturation in human peripheral monocyte-macrophages *in vitro (Reference 1: Enhanced scavenger receptor expression in monocyte-macrophages in dialysis patients. Kidney Int 49:773-780,1996).* In addition, uremic serum enhances SR expression and function in U937 cell line in the standardized culture system *(Reference 2: Ando M, et al: Uremic serum enhances scavenger receptor expression and activity in the human monocytic cell line U937. Kidney Int 51:785-792,1997).* In the latter study, we found out that elevated M-CSF in uremic serum, which is approximately 5-fold higher as compared with healthy serum, could be responsible for this enhancement of SR. Now, we need to further characterize M-CSF in uremic serum and substantiate the effect of M-CSF purified from uremic serum on SR expression. In addition, source or cause of elevated M-CSF in uremic serum should be elucidated.

Materials: uremic serum, healthy serum and peripheral monocytes

Methods: immunoaffinity colum purification, culturing of primary monocyte-macrophage, Western blot, flow cytometry analysis, ELISA.

Results:

1. Molecular species in the uremic serum patterns (Western blot study) were not the same as those in the healthy serum. The fractions of M-CSF less than 50 kDa (native molecular sizes: 70 to 90 kDa) were most strongly existed in the uremic serum and uremic serum included small fractions of M-CSF that were never detected in the healthy one. These findings suggested that the uremic patients have another sauce of the elevated serum M-CSF or the sauce was enhanced in uremic conditions for M-CSF generation..

2. Recombinant M-CSF enhanced M-CSF transcriptional expressions in the monocytic cell line (Northern blot study).

3. On the clinical basis, serum M-CSF levels were statistically significantly correlated with serum intact parathyroid hormone (PTH) levels and those of all parameters of osteoblasts such as intact osteocalcin, alkaliphosohatase derived from bone (ALP III), and P1CP.

Summary 1:

Taken together, we speculated that one of the causes of elevated serum M-CSF is an osteoblast that is strongly stimulate by hyper parathyroidism which is rather common in dialysis patients.

**Research II. Atherosclerosis in uremic mice model: *In vivo* relevance of SR and M-CSF to uremia-associated atherosclerosis**

**Background**: SR expression on macrophage lineage cells could be enhanced in uremic milieu, which might result in the development of atherosclerosis in uremia. This issue was addressed in uremic animal models. Gangnon et al established a uremic mouse model and have shown that uremic C57BL/6J inbred mice fed a normal diet significantly develops atherosclerosis (Urol Res 1988 and Trans Am Soc Artif Intern Organs 1989). Using this model, we could study a systematic lipid metabolism in uremia on the basis of molecular and cell biology.

**Materials**: tissues (bone and peritoneal macrophages) from C57BL/6J with and without uremia.

**Methods**: simple and immunohistological staining, Western blot, ligand blot, Northern blot (or Ribonuclease protection assay), ELISA, flow cytometry analysis.

**Results:**

1. The pathological examination of ascending aorta simply demonstrated that the uremic mice fed a hyperlipidemic diet induced atheroscrelosis around the aortic valves, but not in the mice fed a normal chow This findings indicated the possibility that hyperlipidemic diet induces potential atherosclerosis in uremic condition.
2. The peritoneal macrophages from uremic mice without a hyperlipidemic chow expressed more strongly scavenger receptor mRNA, as compared with those of normal mice. The same results were obtained in protein levels examined by ox-LDL intake, using flow cytometry analysis .
3. Serum M-CSF levels in uremic mice that was measured with ELISA was slightly

elevated but their difference between uremic and control mice was not significant.

4. Recombinant human PTH administered s.c. significantly raised mRNA levels of M-CSF in femur of control mice.

**Summary 2:**

Our data suggested that uremic condition itself accelerated atherosclerosis and under a hyperlipidemic diet. The crosstalk among SR expression, M-CSF, and atherosclerosis is not clear as yet in this study. In addition, *in vivo* secondary hyperparathyroidism observed in uremic patients though, exogenous PTH may elevate M-CSF from bone, leading to the elevation of serum levels of M-CSF.

**Conclusions:**

In uremic mice, scavenger receptor expressions of peritoneal macrophages are enhanced, possibly resulting in accelerated atherosclerosis under a hyperlipidemic chow. This enhancement of scavenger receptors may be induced by elevated M-CSF derived from osteoblasts that are stimulated by secondary hyperparathyroidism in uremia.

## おわりに

慢性腎不全患者は毎年約1万人づつ増加しており、この傾向はここ数年不変であり、現時点で我が国における腎不全患者の総数は、日本透析医学会統計調査委員会のデータによれば、約18万人にも達する。透析医療は高額な医療費と医療技術を必要とする治療であるが、多くの患者が透析を受けながらも必ずしも快適な生活を過ごせているとは限らない。その原因の多くは心血管系の動脈硬化性疾患の合併に依存していると言っても過言ではあるまい。当該研究は、その一端の解明に関わるものであり、今後もこの分野におけるさらなる研究の継続を必要としている。