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〔Short Report〕

Effect of Kurozu (Brewed rice vinegar) on Neovascularization in Rat Ischemic Myocardial Tissue

Noriaki WAKANA*, Kazuhiro HOMMA*, Koji KIMURA**, Naoto FUKUYAMA**, Kazunori MYOJIN**, Masanobu NAGANO***, Etsuro TANAKA*

Kurozu is a traditional brewed rice vinegar that is made in Japan. During the fermentation of Kurozu liquid a solid residue called Kurozu Moromi powder is also produced. We evaluated the effect of Kurozu liquid and Kurozu Moromi powder on neovascularization in ischemic myocardial tissue in the rat. After myocardial ischemia was created by a ligation of the coronary artery, 18 rats consisting of three groups of 6 rats each were fed with the control food, food with the addition of Kurozu liquid, and food with the addition of Kurozu Moromi powder, respectively. Fourteen days after inducing ischemia, the heart was dissected out and the number of vessels/microscopic observation field in the ischemic myocardial tissue was counted by immunohistochemical staining with anti-CD31 antibody. The number of vessels was found to have significantly increased in the both rats that consumed the Kurozu liquid (11.0±3.0/field, 6 rats) and by Kurozu Moromi powder (13.7±2.0/field, 6 rats) in comparison to the control (4.0±1.6/field, 6 rats, $p<0.05$ ANOVA). These results suggest that Kurozu liquid and Kurozu Moromi powder may ameliorate chronic ischemia by promoting neovascularization.

Key words : heart, ischemia, myocardial infarction, rice vinegar, vessels

I. Introduction

Kurozu is a brewed rice vinegar fermented for many years in earthenware jars by traditional methods in Japan[1,2]. The supernatant is known as Kurozu (Kurozu liquid), which is not a simple vinegar but contains various amino acids, vitamins, polyphenols and other organic materials[2,3]. During the fermenting process of Kurozu liquid, a solid residue is produced, called Kurozu Moromi powder, which is rich in organic materials and minerals. Many biological functions of Kurozu liquid, such as improvements in the lipid metabolism[4], allergic reactions[5], hypertension[4], blood fluidity[6], antioxidative activity[3,7], and antitumor promoting activity[7-10] have been reported. However, the effect of Kurozu liquid on the amelioration of ischemia has not yet been examined. Similarly, the effect of Kurozu Moromi powder on diseases including ischemia has also not yet been examined. As a result, we herein evaluated the effect of Kurozu liquid and Kurozu Moromi powder on the neovascularization in ischemic myocardial tissue in the rat.

* Department of Nutritional Sciences, Tokyo University of Agriculture
** Department of Physiology, Tokai University School of Medicine
*** Sakamoto Brewing Co. LTD.

II. Methods

Eighteen male Fisher rats (8 weeks old, CLEA Japan,

Inc., Tokyo) were used. All procedures were carried out in conformity with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No 86-23, revised 1985).

Myocardial ischemia was created by a ligation of the coronary artery in the heart under general anesthesia with isoflurane. Briefly, a left thoracotomy was performed in the fifth intercostal space and then the pericardium incised. The left anterior descending coronary artery was ligated by 10-0 silk thread just below the branching of the left circumflex artery. After the ligation, we confirmed the creation of ischemia by the color change of the left anterior wall of the ventricle and by the appearance of Q waves in electrocardiograms recorded from electrodes placed on the left anterior wall. Thereafter, we sutured the incision of the thoracic wall.

We used 3 different types of food, namely, a control diet (CLEA CE-2, CLEA Japan, Inc., Tokyo), a mixture of CLEA CE-2 (99.68%) and Kurozu liquid (10-folds concentrated, 0.32%), and a mixture of CLEA CE-2 (98%) and Kurozu Moromi powder (2%). After creating ischemia in 18 rats, 6 rats were fed with CLEA CE-2 (the control group), 6 rats with a mixture of CLEA CE-2 and Kurozu liquid (the kurozu group), and 6 rats with a mixture of CLEA CE-2 and Kurozu Moromi powder (the moromi group) ad libitum for 14 days.

The heart was dissected out after the intraperitoneal injection of pentobarbital (150 mg/100 g) on the post operative day 14. Ischemic myocardial tissue was cut out from the anterior free wall of the left ventricle beside the septum at the level of the papillary muscles and frozen for a histological analysis.

Immunohistochemical staining was performed using an indirect immunoperoxidase method. Anti-CD31 antibody (Serotec, UK) was used as the primary antibody. Anti-Ig, peroxidase-linked species-specific F (ab')2 fragments (Amersham Pharmacia Biotech UK Ltd., UK), were used as a secondary antibody. Double staining was performed with alkaline staining and peroxidase staining. The number of vessels stained with anti-CD31 antibody in one microscopic observation field was counted in 16 randomly selected fields for each rat heart.

All data are presented as the mean values±SD. Differences between the means were assessed by ANOVA with the Scheffé F-test. A value of $P<0.05$ was considered to indicate statistical significance.

III. Results

The kurozu and the moromi groups showed neovascularization in the ischemic myocardial tissue. In **Fig. 1**, the vessel walls are stained by the Immunohistochemical method using anti-CD31 antibody. The number of vessels significantly increased in both the kurozu group (11.0±3.0/field, 6 rats) and in the moromi group (13.7±2.0/field, 6 rats) in comparison to the control group (4.0±1.6/field, 6 rats, $p<0.05$ ANOVA) as shown in Fig. 2.

Fig. 1 A histological examination of rat ischemic myocardial tissue. Endothelial cells are stained by immunohistochemical methods with anti-CD31 antibody. The coronary artery was ligated 14 days previously. The rats were fed either the control diet (A), feed with Kurozu liquid (B), or feed with Kurozu Moromi powder (C). Original magnification x400 ; bar= 100μm.

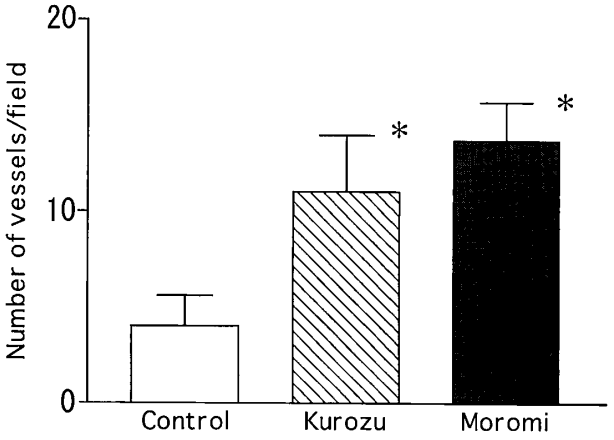

Fig. 2 Effect of Kurozu liquid (Kurozu) and Kurozu Moromi powder (Moromi) on neovascularization in rat ischemic myocardial tissue. The rats were fed the control diet, feed with Kurozu liquid, or feed with Kurozu Moromi powder. The number of vessels were counted using immunohistochemical methods with anti-CD31 antibody 14 days after a ligation of the coronary artery. The results are expressed as the means±SD. *$P<0.05$ vs control (ANOVA)

IV. Discussion

We demonstrated that both Kurozu liquid and Kurozu Moromi powder promoted neovascularization in ischemic myocardial tissue (**Figs. 1** and **2**). In these experiments, we followed the rats for 14 days after inducing ischemia. The period of 14 days is a chronic stage in mice. Neovascularization is a type of endothelial cell proliferation. Kurozu liquid promoted hepatocyte proliferation[11]. Therefore, Kurozu might be effective for not only patients with chronic myocardial infarction but also for patients with chronic ischemia, such as brain ischemia and leg ischemia caused by arteriosclerosis and diabetes mellitus.

The two substances, Kurozu liquid and Kurozu Moromi powder, both showed similar effects. These two substances do not contain the same major ingredients. The key ingredient for neovascularization in these two substances might be different. Acetic acid is the major ingredient of Kurozu liquid. Although acetic acid is volatile, it is preserved in animal feed, namely the mixture of CLEA CE-2 and Kurozu liquid. As a result, acetic acid might thus be the ingredient that causes neovascularization. Kurozu liquid has been reported to show stronger effect than pure acetic acid on both the radical scavenging activity[7] and blood fluidity[6]. The key substance of the former is soluble in ethyl acetate but it not yet been identified[7], while the key ingredient of the latter has been identified to be histamine[6]. Kurozu Moromi powder, which contains less acetic acid, also stimulated neovascularization to a similar extent as that of Kurozu liquid. Therefore, acetic acid might not cause neovascularization. However, we did not identify the ingredients in Kurozu liquid and Kurozu Moromi powder which caused neovascularization nor we did not examine the pure acetic acid in this study.

The contents of Kurozu liquid in the diet was finally calculated to be 3.2%. According to this experimental diet, the rats consumed approximately 1 m*l*/kg/day of Kurozu liquid that was given ad libitum, which corresponded to approximately 50–60 m*l*/day in human in terms of body weight. In the moromi group, the rats consumed approximately 0.7 g/kg/day, which corresponded to 40 g/day in human.

The animal model used in this study was an ischemic heart disease model. Generally speaking, arteries have collateral branches in common tissue and there is a great difference in the density of the collateral network among each animal. For example, since the arteries in the mouse leg have a dense collateral network, a simple ligation of the femoral artery cannot create a stable ischemic model[12]. The coronary artery in mice has sparse collateral vessels. Therefore, a ligation of the coronary artery could create a stable ischemic model, which was thus considered to be suitable as an experimental ischemic model.

In conclusion, both Kurozu liquid and Kurozu Moromi powder promoted neovascularization in the ischemic myocardial tissue and it may therefore ameliorate chronic ischemia.

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References

1) Nanda K, Taniguchi M, Ujike S, Ishihara N, Mori H, Ono H, Murooka Y : Characterization of acetic acid bacteria in traditional acetic acid fermentation of rice vinegar (komesu) and unpolished rice vinegar (kurosu) produced in Japan. Appl Environ Microbiol 67, 986-990, 2001
2) Haruta S, Ueno S, Egawa I, Hashiguchi K, Fujii A, Nagano M, Ishii M, Igarashi Y : Succession of bacterial and fungal communities during a traditional pot fermentation of rice vinegar assessed by PCR-mediated denaturing gradient gel electrophoresis. Int J Food Microbiol 109, 79-87, 2006
3) Shimoji Y, Tamura Y, Nakamura Y, Nanda K, Nishidai S, Nishikawa Y, Ishihara N, Uenakai K, Ohigashi H : Isolation and identification of DPPH radical scavenging compounds in Kurosu (Japanese unpolished rice vinegar). J Agric Food Chem 50, 6501-6503, 2002
4) Tanizawa H, Sazuka Y, Komatsu A, Takino Y : Acute toxicity of Komezu and its effects on lipid metabolism in male mice. Nippon Eiyo Shokuryo Gakkaishi 36, 283-289, 1983 (in Japanese)
5) Nakashima T, Tsutsui I, Nishitani H, Kurimoto Y, Ikenoue T : The effect of a rice vinegar residue on allergic disease (I) Improvement of the uremic pruritus of the patients on chronic hemodialysis. Kiso to Rinshou 25, 2529-2533, 1991 (in Japanese)
6) Yamagishi K, Kimura S, Kameyama M, Nagata T, Kikuchi Y : Purification and identification of blood fluidity improvement factor in brewed rice vinegar (Kurosu). Nippon Shokuhin Kagaku Kogaku Kaishi 45, 545-549, 1998 (in Japanese)
7) Nishidai S, Nakamura Y, Torikai K, Yamamoto M, Ishihara N, Mori H, Ohigashi H : Kurosu, a traditional vinegar produced from unpolished rice, suppresses lipid peroxidation in vitro and in mouse skin. Biosci Biotechnol Biochem 64, 1909-1914, 2000
8) Shimoji Y, Sugie S, Kohno H, Tanaka T, Nanda K, Tamura Y, Nishikawa Y, Hayashi R, Uenakai K, Ohigashi H : Extract of vinegar "Kurosu" from unpolished rice inhibits the development of colonic aberrant crypt foci induced by azoxymethane. J Exp Clin Cancer Res 22, 591-597, 2003
9) Shimoji Y, Kohno H, Nanda K, Nishikawa Y, Ohigashi H, Uenakai K, Tanaka T : Extract of Kurosu, a vinegar from unpolished rice, inhibits azoxymethane-induced colon carcinogenesis in male F344 rats. Nutr Cancer 49, 170-173, 2004
10) Nanda K, Miyoshi N, Nakamura Y, Shimoji Y, Tamura Y, Nishikawa Y, Uenakai K, Kohno H, Tanaka T : Extract of vinegar "Kurosu" from unpolished rice inhibits the proliferation of human cancer cells. J Exp Clin Cancer Res 23, 69-75, 2004
11) Fujii M, Hou D-X, Arimura M, Chiwata T, Suzuki M, Nagano M : Effect of Kurozu (brewed rice vinegar) on maintenance of primary culture of rat hepatocytes. Food Sci Technol Res 5, 97-98, 1999
12) Goto T, Fukuyama N, Aki A, Kanabuchi K, Kimura K, Taira H, Tanaka E, Wakana N, Mori H, Inoue H : Search for appropriate experimental methods to create stable hind-limb ischemia in mouse. Tokai J Exp Clin Med 31, 118-122, 2006

虚血心筋における黒酢の血管新生効果

若菜宣明*・本間和宏*・木村剛爾**・福山直人**・
明神和紀**・長野正信***・田中越郎*

黒酢は日本の伝統的製法により作られた食酢の一種である。醸造中に黒酢もろみ末と呼ばれる固形成分も生成される。本研究は黒酢と黒酢もろみ末とをラットに与え，虚血心筋における血管新生効果を調べた。ラット 18 匹に冠動脈を結紮することにより心筋に虚血を作成した。黒酢群，もろみ群，対照群の 3 群に分け，黒酢群には黒酢（上清）入りの餌，もろみ群には黒酢もろみ末入りの餌，対照群には標準の餌を与えて飼育した。餌は自由摂取とした。14 日後に心臓を摘出し，虚血心筋における血管新生を抗 CD31 抗体を用い免疫組織学的に評価した。顕微鏡 1 視野当りの虚血部の血管数を定量すると，黒酢群（11.0±3.0 本，n＝6）およびもろみ群（13.7±2.0 本，n＝6）の方が対照群（4.0±1.6 本，n＝6，$p<0.05$ ANOVA）よりも有意に多かった。黒酢群ともろみ群との間には差を認めなかった。すなわち，黒酢群およびもろみ群において対照群よりも有意に強い血管新生を認め，黒酢と黒酢もろみ末は心筋虚血部における血管新生を促進させることが明らかとなった。以上の結果より，黒酢はその血管新生作用により虚血性心疾患等の虚血病変に対しその改善効果もしくは病状進行の抑止効果が期待できると考えられた。

キーワード：心臓，虚血，心筋梗塞，酢，血管

* 東京農業大学応用生物科学部栄養科学科
** 東海大学医学部生理学
*** 坂元醸造株式会社