Original Article

Serum copper, zinc and risk factors for cardiovascular disease in community-living Japanese elderly women

Ayaka Tsuboi NRD[1], Mayu Terazawa (Watanabe) NRD[1], Tsutomu Kazumi MD, PhD[1-4], Keisuke Fukuo MD, PhD[1-3]

[1]*Postgraduate School of Food Sciences and Nutrition, Mukogawa Women's University, Hyogo, Japan*
[2]*Department of Food Sciences and Nutrition, School of Human Environmental Sciences, Mukogawa Women's University, Hyogo, Japan*
[3]*Research Institute for Nutrition Sciences, Mukogawa Women's University, Hyogo, Japan*
[4]*Diabetes Center, Myodani Hospital, Hyogo, Japan*

社区居住的日本老年女性血清铜和锌与心血管疾病危险因素的关系

背景：铜和锌的血清水平与心血管疾病危险因素之间的关系尚未在亚洲老年人中进行广泛研究。方法：在 202 名自由居住的日本老年女性中检测了血清铜和锌与心血管疾病危险因素的关系。结果：单因素分析显示：高敏 C 反应蛋白(hsCRP)的对数和非高密度脂蛋白胆固醇均与血清铜浓度有关。铜的独立预测因子是 hsCRP 的对数。随着年龄的增长，血清锌的浓度下降。校正年龄之后，血清白蛋白，高密度脂蛋白胆固醇和红细胞与血清锌成正相关，而血清胰岛素和 hsCRP 的对数与血清锌成负相关。在多元逐步回归分析(模型 1)中，血清白蛋白和高密度脂蛋白胆固醇均与血清锌有关。在将清蛋白从模型 1 中剔除的分析中(模型 2)，血清锌的独立决定因素是 hsCRP 的对数(负相关)和总红细胞数。在包括血清肌氨酸酐的分析中(模型 2)，血清肌氨酸酐是除 hsCRP 的对数和总红细胞数之外的又一个决定因素。在用估计的肾小球滤过率代替肌氨酸酐并排除年龄的分析中(模型 2)，估计的肾小球滤过率是除 hsCRP 的对数和总红细胞数之外的又一个决定因素。结论：老年人全身性低度炎症可能有助于升高血清铜的浓度，降低血清锌的浓度，并可能代表这个人群中血清微量元素和死亡率之间关系的一个重要的混杂因素。

关键词：铜、锌、炎症、女性、老年人

原著2

Determinants of serum uric acid in community-dwelling elderly Japanese women

Ayaka Tsuboi[1)] Mayu Terazawa-Watanabe[2)]
Tsutomu Kazumi[1-4)] Keisuke Fukuo[1-3)]

Aim

Determinants of serum uric acid (SUA) levels in relation to kidney function have not been extensively studied in elderly Japanese people.

Design

In this cross-sectional study, relationships of SUA with anthropometric indices, serum adipokines and liver enzymes, insulin-resistance related variables and inflammatory markers in relation to kidney function were examined in 159 community-dwelling elderly Japanese women.

Results

By simple linear regression analysis, serum creatinine and cystatin C were positively and eGFR was inversely associated with SUA. It was also positively associated with BMI, percentage body fat, serum leptin and transthyretin. In addition, SUA showed positive associations with fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and serum gamma-glutamyltransferase (GGT). Further, log high-sensitivity C-reactive protein (hsCRP) and tumor necrosis factor-alpha (TNF-α) were associated with SUA. Multiple regression analysis with SUA as a dependent variable showed that 27% of the variability of SUA can be accounted for by serum creatinine, GGT, leptin and transthyretin in order of increasing R^2.

Conclusions

In elderly Japanese non-obese women, high serum uric acid levels may represent a more favorable nutritional status and increased body fat in addition to poorer renal function. Associations with serum creatinine and GGT warrant further study.

High serum uric acid (SUA) is one of the best independent predictors of type 2 diabetes and commonly precedes the development of both insulin resistance and diabetes.[1)] An elevated SUA also independently predicts the development of fatty liver,[2)] obesity,[3)] hypertension[4)] and elevations in C-reactive protein (CRP).[5)] Furthermore, metabolic syndrome is associated with a high frequency of hyperuricemia, and similarly, hyperuricemia is associated with metabolic syndrome.[6, 7)] Many of

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1) Postgraduate School of Food Sciences and Nutrition
2) Department of Food Sciences and Nutrition, School of Human Environmental Sciences
3) Research Institute for Nutrition Sciences, Mukogawa Women's University, 6-46, Ikebiraki-cho, Nishinomiya, Hyogo 663-8558, Japan,
4) Diabetes Center, Myodani Hospital, 2350-2, Aza Nashihara, Myodani-cho, Tarumi-ku, Kobe, Hyogo 655-0852, Japan
Key words : uric acid, elderly women, creatinine, transthyretin, gamma-glutamyltransferase

these studies were conducted mainly in middle-aged and male populations, and correlates of SUA levels have not been extensively studied in elderly Japanese people.

Reduced renal function and high SUA are associated with each other and are common in the elderly.[8, 9] As SUA levels are higher in postmenopausal as compared to premenopausal women,[10, 11] we investigated associations of SUA with serum levels of hepatic enzyme and adipokines, markers of adiposity, insulin resistance, and inflammation, and components of metabolic syndrome in community-dwelling postmenopausal elderly women.

Subjects and Methods

We examined 159 free-living elderly women who have been reported elsewhere.[12] They were residents in Nishinomiya City and were recruited as volunteers by local welfare commissioners from the city of Nishinomiya, Hyogo, Japan. Although 43, 9 and 58 women (27.0, 5.7, and 36.5%, respectively) reported to be receiving statins, anti-diabetic and anti-hypertensive drugs, respectively, detailed drug information was not available. Subjects with clinically diagnosed acute or chronic inflammatory diseases, endocrine, cardiovascular, hepatic, or renal diseases, taking hormonal contraception, or showing unusual dietary habits were excluded from the study. This research followed the tenets of the Declaration of Helsinki. The design of this study was approved by the Ethical Committees of Mukogawa Women's' University and written informed consent was obtained from all participants.

Anthropometric indices and blood pressure were measured after an overnight fasting as previously reported.[12] Thereafter, blood samples were obtained from the cubital vein. Fat mass was measured using an impedance method (InBody 430, Biospace, Tokyo, Japan).

We evaluated routine chemical parameters, including liver enzymes and albumin. Plasma glucose, serum insulin, lipids and lipoproteins were assayed as previously reported[13, 14] and insulin resistance was evaluated using homeostasis model assessment (HOMA-IR).[15] Low-density lipoprotein (LDL) cholesterol was determined using Friedewald's formula.[16] Serum transthyretin (TTR), also called prealbumin, was measured as previously reported.[17]

Adiponectin was assayed with a sandwich enzyme-linked immunosorbent assay (Otsuka Pharmaceutical Co., Ltd., Tokushima City, Japan). Intra- and inter- assay CV were 3.3% and 7.5%, respectively. Leptin was assessed using a RIA kit from LINCO Research (St. Charles, MO,USA, inter-assay CV=4.9%). High-sensitivity CRP (hs-CRP) was measured by an immunoturbidometric assay with the use of reagents and calibrators from Dade Behring Marburg GmbH (Marburg, Germany; interassay CV<5%). Tumor necrosis factor-alpha (TNF-α) was measured by immunoassays (R&D Systems, Inc., Minneapolis, MN, USA, interassay CV<6%). Plasminogen activator inhibitor-1 (PAI-1) was measured by an ELISA method. (Mitsubishi Chemicals, interassay CV<8%). The complete blood cell count was analyzed using an automated blood cell counter (Sysmex XE-2100, Sysmex, Kobe, Japan).

Serum creatinine and uric acid were measured enzymatically using an Autoanalyzer (AU 5200, Olympus, Tokyo, Japan) and cystatin C was measured by latex immunoassay using a commercially available kit (IatroCys-C, Mitsubishi Chemical Medience, Tokyo, Japan). The estimated glomerular filtration rate (eGFR)

Table 1. Anthropometric and biochemical characteristics of 159 free-dwelling women studied and correlation coefficients of serum uric acid.

	Mean ± SD	serum uric acid	
		simple	partial
Age (years)	75.6 ± 8.0	-.063	-.376 ***
BMI (kg/m^2)	22.6 ± 2.9	.235 **	.234 **
Body fat percentage (%)	33.0 ± 6.9	.171 *	.128
SBP (mmHg)	143.4 ± 19.0	.089	-.018
DBP (mmHg)	84.4 ± 10.4	.070	.038
Albumin (g/dl)	4.4 ± 0.3	-.044	.073
Transthyretin (mg/dl)	28.2 ± 4.8	.185 *	.242 **
ALT (U/l)	19.3 ± 16.6	.111	.200 *
GGT (U/l)	25.0 ± 15.9	.252 **	.299 ***
Plasma glucose (mg/dl)	100 ± 29	.134	.105
Insulin (μU/ml)	8.3 ± 7.5	.160 *	.089
log insulin	0.7 ± 0.2	.213 **	.149
HOMA-IR	1.23 ± 1.08	.163 *	.083
log HOMA-IR	0.002 ± 0.256	.221 **	.158
Total cholesterol (mg/dl)	219 ± 31	-.096	-.023
HDL-cholesterol (mg/dl)	64 ± 14	-.116	-.056
nonHDL-cholesterol (mg/dl)	155 ± 33	-.042	.004
Triglyceride (mg/dl)	119.2 ± 64.9	.003	-.015
Serum uric acid (mg/dl)	4.8 ± 1.0	1.000 ***	1.000 ***
Serum creatinine (mg/dl)	0.69 ± 0.15	.412 ***	.235 **
Cystatin C (mg/l)	0.85 ± 0.20	.343 ***	.043
eGFRaver (ml/min/1.73 m^2)	71.1 ± 15.9	-.348 ***	adjusted
Leptin (ng/ml)	7.7 ± 4.7	.296 ***	.245 **
Adiponectin (μg/ml)	14.1 ± 7.8	-.088	-.154
hsCRP (μg/dl)	85 ± 109	.071	.031
log hsCRP	1.71 ± 0.42	.165 *	.110
TNF-α (pg/ml)	1.6 ± 1.0	.218 **	.066
PAI-1 (ng/ml)	26.5 ± 16.5	.132	.173 *
IL-6 (pg/ml)	5.5 ± 12.0	.133	.077
White blood cells ($\times 10^3/\mu l$)	6.1 ± 1.6	.033	-.043

BMI : body mass index, FMI: fat mass index, GGT : γ-glutamyltransferase, eGFR : estimated glomerular filtration rate, hsCRP : high- sensitivity CRP, TNF-α : tumor necrosis factor-α, PAI-1 : plasminogen activator inhibitor-1, IL-6 : interleukin-6. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

was calculated using the equation recommended by the Japanese Society of Nephrology.[18, 19] The average of creatinine-based and cystatin-C based eGFR was calculated in each participant. Average eGFR has been recommended for estimation of renal function in elderly populations[20] and therefore, was used in statistical analysis in the present study. Reduced kidney function[18, 19] was defined as eGFR<60 ml/min/1.73m^2.

Data are presented as the mean ± SD unless otherwise stated. Due to deviation from a normal distribution, insulin, HOMA-IR and hsCRP were logarithmically transformed for analysis. Differences between 2 groups were analyzed by t test and frequencies of conditions by the Chi-square test. Differences among 3 groups were analyzed using analysis of variance. When p values in analysis of variance were $p<0.05$, Bonferroni's multiple comparison procedure was performed. Correlations of SUA were evaluated by Pearson correlation analysis. Stepwise multiple regression analyses were performed to further indentify the most significant variables contributing to the variation of SUA. Potential confounders or variables of interest were forced into the model and standardized β coefficients were calculated. The explanatory power of the model was expressed as adjusted R^2 values. A two-tailed $p<0.05$ was considered significant. All calculations were performed with SPSS system 15.0 (SPSS Inc, Chicago, IL, USA).

Results

As previously reported,[12] participants were apparently healthy, community-dwelling elderly women (Table 1). SUA averaged 4.8 ± 1.0 mg/dl and SUA ≧ 6.0mg/dl[10] was found in 22 women (13.8%) whereas there were only 3 women (1.9%) with SUA ≧ 7.0 mg/dl. Of 159 women, 104 (65.4%) had hypertension (on anti-hypertensive medication or systolic blood pressure (SBP)/diastolic blood pressure (DBP) ≧ 140/90 mmHg without medication) and SBP/DBP averaged 143/84mmHg. In contrast, obesity (BMI ≧ 25.0 kg/m^2) was found in only 16.4% (26 women) and the average BMI was 22.6 kg/m^2. Reduced kidney function was noted in 32 women (20.1%).

In simple linear regression analysis (Table 1), SUA was associated positively with serum creatinine and cystatin C, and inversely with average eGFR. It was positively associated with body fat percentage, serum leptin, HbA1c, log insulin and log HOMA-IR. In addition, SUA was associated with log hsCRP and TNF-α. Furthermore, it was associated with TTR and GGT. However, SUA was not associated with serum triglycerides, HDL cholesterol or blood pressure. After adjustment for average eGFR (Table 1), the association with creatinine remained significant although that with cystatin C turned to be non-significant. Associations with age, alanine aminotransferase (ALT) and PAI-1 turned to be significant. However, association with age was not positive but inverse. Although associations with log insulin, log HOMA-IR, log hsCRP and TNF-α became insignificant, associations remained significant with BMI, percentage body fat, leptin, HbA1c, GGT and TTR after adjustment for average eGFR.

We have done multiple regression analysis with SUA as a dependent variable, which included average eGFR and all variables that showed significant associations with SUA after adjustment for average eGFR as independent variables (Table 2, model A). Serum creatinine and leptin as well as GGT and TTR were determinants of SUA independently of renal

Table2 Stepwise multiple regression analysis with serum uric acid as dependent variable in community-dwelling elderly womenn

model A	Standardized β	p value	cumulativeR^2
serum creatinine	0.359	<0.001	0.182
GGT	0.193	0.007	0.223
leptin	0.189	0.011	0.255
transthyretin	0.148	0.038	0.272
model B			
serum creatinine	0.361	<0.001	0.182
GGT	0.199	0.006	0.223
leptin	0.198	0.008	0.255

Data are standardized β. Model A included all variables which showed significant associations with uric acid after controlling for average estimated glomerular filtration rate (eGFR) in Table 1; age, BMI, percentage body fat, ALT, GGT, leptin, PAI-1, transthyretin, HbA1c, creatinine and an average of creatinine-based and cystatin-based eGFR in Table 1. In Model B, all parameters which showed significant differences between women with and without hyperuricemia after controlling for average eGFR were included; age, BMI, percentage body fat, pre-heparin lipoprotein lipase, log hsCRP, GGT, leptin, creatinine and an average of creatinine-based and cystatin-based eGFR.

function and inflammatory markers in community-living elderly women. These 4 variables explained 27.2% of SUA variability.

Because it has been reported that women with SUA≧6.0mg/dl have an increased risk of end-stage kidney disease,[8] elderly women were divided into 2 groups (Table 3). Elderly women with SUA≧6.0mg/dl as compared to women with SUA<6.0mg/dl had higher BMI, body fat percentage and serum leptin. Serum creatinine and cystatin C were also higher and eGFR was lower in women with elevated SUA. Log insulin and log HOMA-IR were marginally higher in women with elevated SUA although there was no difference in blood pressure or the proportion of women on antihypertensive drugs (72.7 and 64.2% of women with and without elevated SUA, respectively, p=0.43). Furthermore, women with elevated SUA had higher GGT, log hsCRP and TNF-α. After controlling for average eGFR, the difference in age turned to be significant: women with SUA≧6.0mg/dl were younger, not older than those with SUA<6.0 mg/dl (70.3 ± 1.4 vs. 76.5±0.5 (standard errors) years old, p<0.001). Differences remained significant for BMI, percentage body fat, leptin, creatinine, eGFR, log hsCRP and GGT although significant differences in log insulin, log HOMA-IR and log TNF-α were abolished (data not shown).

Multiple regression analysis with SUA as a dependent variable and average eGFR and all parameters which showed significant differences between women with and without hyperuricemia after controlling for average eGFR as independent variables (Table 2, model B), showed that 25.5% of the SUA variability can be accounted for by serum creatinine, GGT and leptin in order of increasing R^2.

Table 3. Anthropometric and biochemical characteristics of women with elevated uric acid (≧6.0mg/dl)

	Not elevated			elevated			P-value
	n=137			n=22			
Age (years)	75.8	±	8.0	74.5	±	8.6	0.484
BMI (kg/m^2)	22.2	±	2.8	24.4	±	3.1	0.001
Body fat percentage (%)	32.4	±	6.9	36.4	±	6.0	0.013
SBP (mmHg)	142.9	±	19.2	146.3	±	18.5	0.450
DBP (mmHg)	84.4	±	10.7	84.7	±	8.4	0.899
Albumin (g/dl)	4.4	±	0.3	4.4	±	0.3	0.546
Transthyretin (mg/dl)	28.1	±	4.8	28.7	±	5.1	0.543
ALT (U/l)	18.5	±	9.8	19.6	±	10.7	0.623
GGT (U/l)	23.4	±	14.6	34.5	±	19.9	0.019
Plasma glucose (mg/dl)	87	±	13	89	±	14	0.488
Insulin (μU/ml)	5.3	±	3.6	6.9	±	6.1	0.085
log insulin	0.66	±	0.23	0.76	±	0.23	0.050
HOMA-IR	1.18	±	0.98	1.56	±	1.56	0.135
log HOMA-IR	-0.013	±	0.256	0.100	±	0.244	0.054
Total cholesterol (mg/dl)	223	±	32	208	±	34	0.049
HDL-cholesterol (mg/dl)	67	±	16	62	±	15	0.196
nonHDL-cholesterol (mg/dl)	156	±	32	146	±	30	0.164
Triglyceride (mg/dl)	120.3	±	67.0	112.0	±	50.9	0.582
Serum uric acid (mg/dl)	4.5	±	0.8	6.6	±	0.4	0.000
Serum creatinine (mg/dl)	0.70	±	0.15	0.87	±	0.17	0.000
Cystatin C (mg/l)	0.83	±	0.19	0.99	±	0.23	0.001
eGFRmean ($ml/min/1.73m^2$)	73.0	±	15.5	59.1	±	12.5	0.000
Leptin (ng/ml)	8.6	±	5.8	12.7	±	7.5	0.003
Adiponectin (μg/ml)	16.0	±	7.5	14.9	±	7.0	0.548
hsCRP (μg/dl)	193	±	335	339	±	529	0.222
log hsCRP	1.84	±	0.59	2.18	±	0.53	0.013
TNF-α (pg/ml)	0.3	±	0.2	0.4	±	0.2	0.007
PAI-1 (ng/ml)	28.8	±	11.3	30.2	±	8.5	0.570
IL-6 (pg/ml)	4.7	±	6.0	10.5	±	28.7	0.360
White blood cells ($\times 10^3/\mu l$)	5.8	±	1.3	6.0	±	2.0	0.633

Data are mean ± SD. Abbreviations are the same as in Table 1.

Table 4. Anthropometric and biochemical characteristics of women according to tertiles of serum uric acid.

	Low	Medium	High
	2.5-4.2	4.3-5.0	5.1-7.6
	n=50	n=52	n=57
Age (years)	76.6 ± 7.1	74.3 ± 8.7	76.0 ± 8.2
BMI (kg/m^2)	22.2 ± 2.5[a]	21.8 ± 2.9[a]	23.5 ± 3.1[b]
Body fat percentage (%)	32.2 ± 6.6[a]	31.5 ± 6.7[a]	35.0 ± 6.9[b]
SBP (mmHg)	141.1 ± 18.1	143.9 ± 18.7	144.9 ± 20.4
DBP (mmHg)	83.3 ± 9.8	85.3 ± 11.3	84.6 ± 10.1
Albumin (g/dl)	4.4 ± 0.3	4.5 ± 0.2	4.4 ± 0.3
Transthyretin (mg/dl)	26.7 ± 4.3[a]	28.8 ± 5.1[b]	28.9 ± 4.8[b]
ALT (U/l)	16.6 ± 7.0	19.0 ± 9.2	20.1 ± 12.3
GGT (U/l)	20.9 ± 9.6[a]	22.6 ± 9.6[a]	30.7 ± 22.2[b]
Plasma glucose (mg/dl)	84.9 ± 7.7	89.7 ± 10.8	88.3 ± 17.0
Insulin (μU/ml)	5.2 ± 4.5	5.0 ± 2.4	6.2 ± 4.7
log insulin	0.63 ± 0.25[a]	0.65 ± 0.20[ab]	0.72 ± 0.23[b]
HOMA-IR	1.12 ± 1.06	1.12 ± 0.59	1.43 ± 1.40
log HOMA-IR	-0.052 ± 0.272[a]	-0.003 ± 0.217[ab]	0.055 ± 0.268[b]
Total cholesterol (mg/dl)	222.5 ± 29.6[ab]	227.5 ± 29.7[a]	212.6 ± 26.8[b]
HDL-cholesterol (mg/dl)	68.5 ± 16.2	65.8 ± 13.3	65.0 ± 17.1
nonHDL-cholesterol (mg/dl)	153.9 ± 24.5[ab]	161.8 ± 30.4[a]	147.6 ± 36.9[b]
Triglyceride (mg/dl)	121.5 ± 73.1	120.9 ± 63.1	115.5 ± 59.7
Serum Uric acid (mg/dl)	3.6 ± 0.5[a]	4.7 ± 0.2[b]	5.9 ± 0.6[c]
Serum creatinine (mg/dl)	0.66 ± 0.10[a]	0.70 ± 0.15[a]	0.81 ± 0.18[b]
Cystatin C (mg/l)	0.77 ± 0.11[a]	0.85 ± 0.22[a]	0.93 ± 0.22[b]
eGFRmean (ml/min/1.73m^2)	76.8 ± 15.4[a]	73.6 ± 17.0[a]	63.9 ± 14.9[b]
Leptin (ng/ml)	7.4 ± 4.3	9.0 ± 5.7	10.9 ± 7.5
Adiponectin (μg/ml)	16.3 ± 6.4	16.6 ± 9.2	14.7 ± 6.2
hsCRP (μg/dl)	230.9 ± 338.6	163.8 ± 377.5	243.6 ± 388.0
log hsCRP	1.821 ± 0.698	1.804 ± 0.509	2.010 ± 0.556
TNF-α (pg/ml)	1.98 ± 0.91[a]	2.41 ± 1.08[ab]	2.52 ± 1.57[b]
PAI-1 (ng/ml)	27.4 ± 9.7	28.7 ± 11.4	30.7 ± 11.4
IL-6 (pg/ml)	3.89 ± 4.25	5.83 ± 8.03	6.66 ± 18.12
White blood cells (×10^3/μl)	5.7 ± 1.3	5.7 ± 1.4	6.0 ± 1.6

Data are mean ± SD. Abbrevations are the same as inTable 1. Means not sharing common letters are significantly different each other at $p<0.05$ or less

In order to further confirm the correlations of SUA, elderly women were divided into 3 groups according to the tertiles of SUA (Table 4). Women in the top tertile had higher BMI, percentage body fat and log GGT than those in the other 2 groups. The prevalence of women on anti-diabetic medication was higher in the top (n=8, 14.0%) compared to the median and bottom SUA tertiles (n=1, 1.9 and 0%, respectively, p=0.003). In addition, HbA1c, log insulin, log HOMA-IR and log ALT were higher in women in the top as compared to the lowest SUA tertile. Furthermore, women in the median and top as compared to the lowest SUA tertile had higher TTR and log TNF-alpha Associations with BMI, TTR, HbA1c, GGT and ALT remained significant after controlling for average eGFR (data not shown). There was no difference in SBP, DBP and lipid and lipoprotein levels or the prevalence of women receiving statin and anti-hypertensive drugs.

Discussion

To the best of our knowledge, this is the first report assessing the relationship of SUA with anthropometric indices, serum adipokines, insulin-resistance related, inflammatory and hematological variables in elderly Japanese women. The present studiy demonstrated that higher SUA was associated with higher serum levels of creatinine, GGT, leptin and TTR in elderly Japanese women. These associations were independent of renal function, insulin resistance and inflammation. We confirmed the previous findings that high SUA is associated with reduced renal function[8, 9] and high serum leptin levels.[21, 22] It is noteworthy that these findings were observed in community-dwelling elderly women who had fewer indicators of disease, such as a low BMI, hypoalbuminemia and hypocholesterolemia.

Serum creatinine was the strongest correlate of SUA independent of kidney function in elderly Japanese women as previously reported in middle-aged Japanese males.[23] Baseline concentrations of, and 10 year changes in SUA were associated with baseline concentrations of, and changes in serum creatinine in young women.[24] Although underlying mechanisms of these associations remain unclear, it has been suggested that the skeletal muscles, a source of serum creatinine, may be an important source of uric acid in the circulation in hypertensive patients.[25] Hypertension was observed in 104 (65.4%) of the 159 women studied in the present study.

While uric acid has pro-oxidative effects inside the cell, it is an antioxidant in the extracellular environment.[1] Recently, serum GGT concentrations have become regarded as a marker of oxidative stress.[26] A recent epidemiological study in Japanese men showed that increased serum GGT is an independent predictor of the subsequent development of hyperuricemia.[27] Taken together, these findings may provide a rationale for the positive association between GGT and SUA and suggest that oxidative stress may play a key role in this relationship.[27]

An elevated uric acid cross-sectionally is associated with BMI[28] and independently predicts future weight gain or obesity.[3] As previously reported in obese or diabetic people[29, 30] and non-obese Japanese middle-aged women,[31] and confirmed in the present study of elderly non-obese women, serum leptin, a marker of the amount of adipose tissue in the body,[32] was a determinant of SUA independent of BMI, percentage body fat, kidney function and insulin resistance (HOMA-IR). Although

the underlying mechanisms of the association between SUA and leptin remained unclear, Bedir A et al.[29] recently discussed the role of leptin as possibly being a regulator of SUA concentrations in humans and suggested that leptin might be one of the possible candidates for the missing link between obesity and hyperuricemia.

Because TTR is a useful marker of malnutrition in the elderly,[33] a significant and independent association between TTR and SUA in the present study may represent more favorable nutrition in elderly women with higher SUA. However, it is interesting that 5-hydroxyisourate hydrolase, another enzyme participating urate degradation to allantoin,[34] and TTR, a carrier protein involved in the extracellular transport of thyroid hormones and in the co-transport of retinol, are closely related phylogenetically and structurally.[35] It has been shown that a small number of critical mutations affecting the active site of the enzyme may be sufficient to generate the markedly different function.[35]

Adjustment for kidney function abolished the association of SUA with fasting insulin and HOMA-IR, and with inflammatory markers in the current study. The former may be in accordance with data suggesting that renal insufficiency suppresses renal clearance of insulin, leading to its higher circulating levels.[36] The latter finding may be in line with the observation that inflammatory biomarkers are elevated in elderly persons with renal insufficiency.[37]

Elevated SUA has been shown to be associated with low-grade inflammation in Caucasian population-based study [38] and Italian elderly cohort.[39] However, decreases in SUA with accompanying increased urinary excretion of uric acid were associated with 2 conditions of clinically overt inflammation: during gouty attack [40] and after the exogenous administration of human recombinant IL-6 in cancer patients [41] A modest elevation of IL-6 may be a consequence of modest increases in SUA in the former [5, 38, 39] whereas markedly elevated IL-6 may be a cause of a decrease in SUA in the latter.[40, 41]

In 2487 middle-aged Japanese men,[23] serum creatinine was the strongest correlate of SUA, followed by serum TG, BMI and diastolic blood pressure. In 916 Japanese elderly women,[42] with increasing SUA, TG levels and diastolic blood pressure increased and HDL cholesterol decreased even in the absence of metabolic syndrome. In the present study, however, we did not find associations with serum lipids and blood pressure. This may be in part due to small sample size in our study.

Several limitations must be acknowledged. The cross-sectional design of this study does not allow for the identification of causal relationships. The recruitment procedure may also have had an impact on the results. As participation was voluntary, women who pay more attention to their health may have been more likely to participate. Biochemical parameters, including SUA levels, were measured only once. Although 58 women (36.5%) reported to be receiving anti-hypertensive drugs information regarding diuretics was not available; therefore, the possible contribution of diuretics or other drugs to SUA levels [43] or other parameters cannot be completely excluded. Sakaki and Tsuchihasi [44] reported that diuretics were administered in 22% of patients on anti-hypertensive drugs.

In spite of these limitations, the present study is the first to examine determinants of uric acid

in elderly Japanese women. High serum uric acid levels may represent a more favaroble nutritional status and increased body fat in addition to poorer renal function in Japanese postmenopausal women. Associations with serum creatinine and GGT warrant further study.

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SHORT REPORT Open Access

Partial suppression of M1 microglia by Janus kinase 2 inhibitor does not protect against neurodegeneration in animal models of amyotrophic lateral sclerosis

Satoru Tada[1,2], Tatsusada Okuno[1*], Yasumichi Hitoshi[3], Teruhito Yasui[2], Josephe Archie Honorat[1], Kazushiro Takata[1], Toru Koda[1], Hiroshi Shimagami[1], Choong Chi-Jing[1], Akiko Namba[1], Tomoyuki Sugimoto[4], Saburo Sakoda[5], Hideki Mochizuki[1], Hitoshi Kikutani[2] and Yuji Nakatsuji[1*]

Abstract

Background: Accumulating evidence has shown that the inflammatory process participates in the pathogenesis of amyotrophic lateral sclerosis (ALS), suggesting a therapeutic potential of anti-inflammatory agents. Janus kinase 2 (JAK2), one of the key molecules in inflammation, transduces signals downstream of various inflammatory cytokines, and some Janus kinase inhibitors have already been clinically applied to the treatment of inflammatory diseases. However, the efficacy of JAK2 inhibitors in treatment of ALS remains to be demonstrated. In this study, we examined the role of JAK2 in ALS by administering a selective JAK2 inhibitor, R723, to an animal model of ALS ($mSOD1^{G93A}$ mice).

Findings: Orally administered R723 had sufficient access to spinal cord tissue of $mSOD1^{G93A}$ mice and significantly reduced the number of *Ly6c positive* blood monocytes, as well as the expression levels of IFN-γ and nitric oxide synthase 2, inducible (iNOS) in the spinal cord tissue. R723 treatment did not alter the expression levels of Il-1β, Il-6, TNF, and NADPH oxidase 2 (NOX2), and suppressed the expression of Retnla, which is one of the markers of neuroprotective M2 microglia. As a result, R723 did not alter disease progression or survival of $mSOD1^{G93A}$ mice.

Conclusions: JAK2 inhibitor was not effective against ALS symptoms in $mSOD1^{G93A}$ mice, irrespective of suppression in several inflammatory molecules. Simultaneous suppression of *anti-inflammatory microglia* with a failure to inhibit critical other inflammatory molecules might explain this result.

Keywords: Amyotrophic lateral sclerosis, SOD1-G93A transgenic mice, R723, Janus kinase 2, JAK2 inhibitor, Neuroinflammation, Interferon gamma, M1/M2 microglia

Findings

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating disease characterized by progressive degeneration of motor neurons in the brain and spinal cord, resulting in muscle weakness. Although the precise mechanism of ALS remains unknown, inflammatory microglial activation plays an important role in pathogenesis [1-3]. The inflammatory molecule IFN-γ, which is primarily produced by Th1 lymphocytes and is a potent activating factor for inflammatory M1 microglia, contributes to the loss of motor neurons in ALS [4,5]. Furthermore, a recent report showed that Ly6c-high inflammatory monocytes are recruited to the spinal cord in $mSOD1^{G93A}$ mice, and that treatment with anti-Ly6c monoclonal antibodies reduces monocyte recruitment to the spinal cord and ameliorates neurodegeneration in these animals [6].

Janus kinases (JAKs) are centrally implicated in cytokine receptor-mediated cell signaling pathways, which drive a

* Correspondence: okuno@neurol.med.osaka-u.ac.jp; yuji@neurol.med.osaka-u.ac.jp
[1]Department of Neurology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
Full list of author information is available at the end of the article

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range of myeloid malignancies [7] as well as inflammatory diseases [8]. The JAK family member JAK2 is responsible for transducing signals for several proinflammatory cytokines including IFN-γ and Il-12, as well as for differentiation of myeloid cells [9]. In an animal model of rheumatoid arthritis (RA) and experimental autoimmune encephalitis (EAE), suppression of the JAK pathway ameliorated disease severity by suppressing Th1 cells and deactivating monocytes [10,11]. A growing number of JAK inhibitors have been developed and clinically applied to the treatment of various inflammatory disease including RA, psoriasis, and inflammatory bowel disease [12-14]. Although activators of JAK2 such as IFN-γ, Il-6, and Il-12 are reported to be implicated in ALS pathogenesis [3], the role of JAK2 in the ALS-related neuroinflammation remains totally unknown.

Based on these findings, we hypothesized that JAK2 inhibition could ameliorate neurodegeneration in ALS model mice by inhibiting harmful inflammatory processes in microglia/macrophages. To test this idea, we treated transgenic mice overexpressing the familial ALS-associated G93A SOD1 mutation (mSOD1^{G93A} mice) with R723, an orally active inhibitor of JAK2 [15].

Methods

Ethics statements

All animal experiments were conducted in accordance with the guidelines of Osaka University, which specifically approved this study (Permit number: Biken-AP-H21-28-0).

RNA extraction and RT-qPCR analysis

Spinal cord tissues were collected from mSOD1^{G93A} mice and total mRNA and cDNA were generated as previously described [1]. The synthesized cDNA was amplified using SYBR Premix Ex Taq II (for TNF, MCP1, Il-12b, iNOS, Il-6, Il-1b, NOX2, Ly6c, Arg1, Ym1, Il-4, EPO, CSF3 and Retnla) (Takara Bio Inc., Otsu, Japan) or TaqMan Gene Expression Assays (for IFN-γ, Il-6, Il-12a and GM-CSF) (Applied Biosystems, Foster City, CA, USA) and analyzed as previously described [1].

Immunohistochemistry

Spinal cord sections of mSOD1^{G93A} mice were prepared as previously described [1]. The following antibodies were used: rabbit anti-JAK2 (phospho Y1007 + Y1008) monoclonal antibody (1:200; Abcam, Cambridge, UK), rabbit anti-iNOS polyclonal antibody (1:50; BD Biosciences, Franklin Lakes, NJ, USA) and Alexa Fluor 488®-conjugated mouse anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (1:200; Cell Signaling Technology, Beverly, MA, USA). The following secondary antibodies were applied: Cy5-conjugated F(ab') 2 fragment donkey anti-rabbit IgG (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA, USA).

Animals and R723 administration

mSOD1^{G93A} mice were obtained from The Jackson Laboratory and backcrossed with C57BL/6 mice for at least 10 generations. R723 was administered by oral gavage starting on day 90. For the analysis of motor function by rotarod test, weight measurement, and survival, R723 dosing continued until day 120 (70 mg/kg twice daily; 5 days on, 2 days off). To evaluate *in vivo* pharmacokinetics, plasma and spinal cord tissues were collected at 0.5, 1, 2, and 4 hours post-dose, and R723 levels in plasma and spinal cord tissue were determined by LC/MS/MS.

Flow cytometry of peripheral blood cells

Peripheral blood cells were collected from mSOD1^{G93A} mice on day 4 post-dose. The following antibodies were used: APC-Cy7-labeled anti-CD11b (M1/70; BioLegend, San Diego, CA, USA) and fluorescein isothiocyanate (FITC)-labeled anti-Ly6c (HK1.4; BioLegend, San Diego, CA, USA). Flow cytometry was performed using a FACS Canto™ II with the Diva ™ software (Becton Dickinson, Franklin Lakes, NJ, USA). Acquired data were analyzed using the FlowJo software (Tree Star, Inc., Ashland, OR, USA).

Lectin staining

Sections were permeabilized with 0.2% tris-buffered saline with tween (TBST) for 10 minutes and then incubated with FITC-conjugated tomato (*Lycopersicon esculentum*) lectin (Sigma-Aldrich, St Louis, MO, USA) diluted 1:750 in PBS overnight at 4°C. The sections were washed ×3 in 0.2% TBST for 5 minutes and mounted with VECTASHIELD Mounting Medium containing 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA). The fluorescently labeled sections were examined using a LSM 510 confocal microscope (Carl Zeiss Microscopy, Jena, Germany).

Nissl staining

Spinal cord sections of mSOD1^{G93A} mice were prepared as previously described [1]. Every fifth section was collected and stained with cresyl violet.

Statistics

Data are expressed as means ± SEM. Differences in animal weight measurements and rotarod tests were assessed using analysis of variance (ANOVA). Statistical significance in survival experiments was determined using Kaplan-Meier survival statistics. Statistical significance in all other experiments was assessed using the Mann-Whitney *U*-test. $P < 0.05$ was considered statistically significant.

Results

To confirm whether expression of inflammatory cytokines was upregulated in the spinal cords of late-stage mSOD1^{G93A} mice, we evaluated spinal cord mRNA expression of several genes encoding inflammatory molecules. Consistent with a previous report [16], RT-qPCR analysis revealed that the expression levels of IFN-γ, Il-6, Il-12a, and granulocyte macrophage colony-stimulating factor (GM-CSF) increased along with disease progression (Figure 1A and Additional file 1: Supplementary information). In addition, microglia in the spinal cords of late stage mSOD1^{G93A} mice (130 days old) had enhanced phosphorylation of JAK2 compared with pre-onset stage mSOD1^{G93A} mice (70 days old), providing a therapeutic rationale for JAK2 inhibition against ALS (Figure 1B, C).

To investigate the role of JAK2 pathway in ALS, we used R723, which is a selective small-molecule JAK2 inhibitor originally developed by Rigel Pharmaceuticals Inc, (San Francisco, CA, USA) for the treatment of myeloproliferative neoplasms such as polycythemia vera, essential

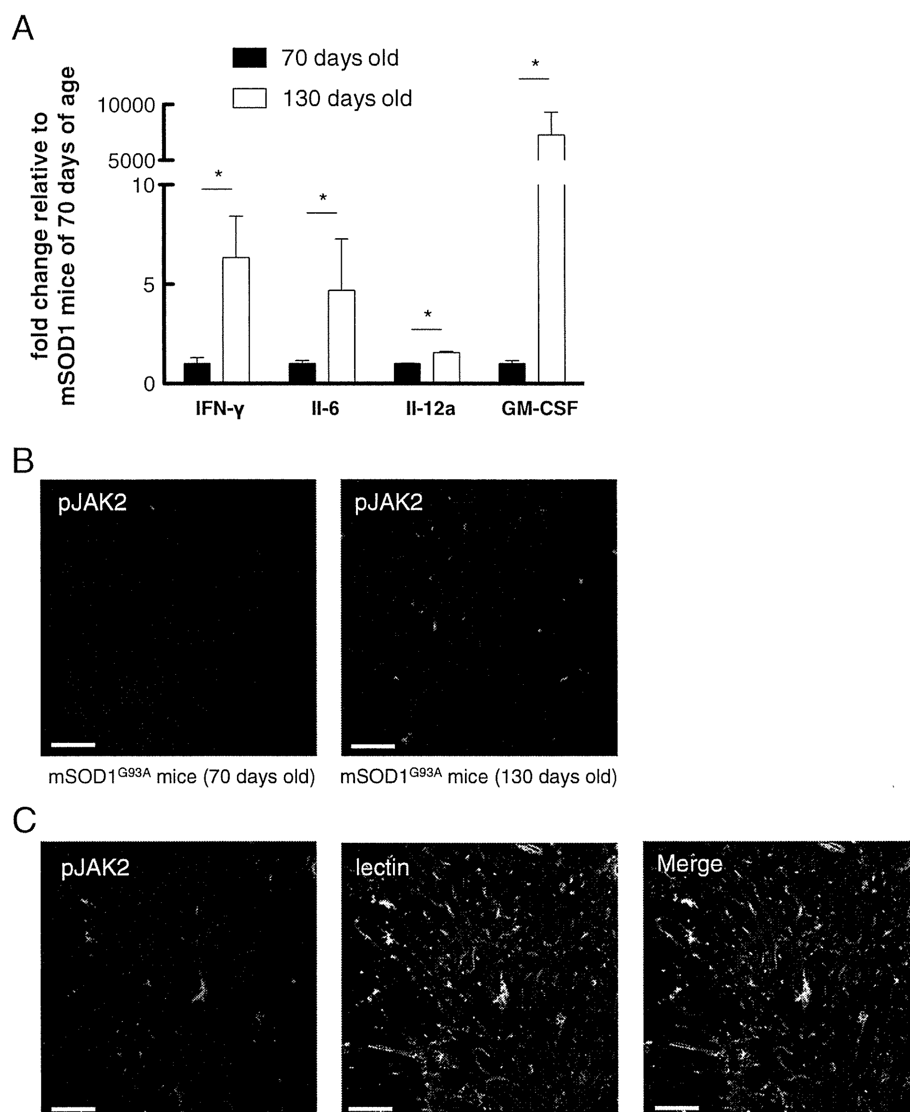

Figure 1 Enhanced phosphorylation of Janus kinase 2 (JAK2) and up-regulation of JAK2-related genes in the spinal cord of mSOD1^{G93A} mice in the late stage of disease. **(A)** Quantitative RT-PCR analyses of spinal cords of mSOD1^{G93A} mice (70 days and 130 days old) were performed (n = 3 to 4 for each group). Expression levels of IFN-γ, Il-6, Il-12a, and GM-CSF were significantly elevated in 130-day-old mSOD1^{G93A} mice relative to those in 70-day-old mice. Data are expressed as means ± SEM. *$P < 0.05$, Mann-Whitney *U*-test. **(B)** Immunohistochemical analysis showed enhanced phosphorylation of JAK2 in the spinal cord of late-stage mSOD1^{G93A} mouse compared with the spinal cord of pre-onset-stage mSDO1 mouse. Scale bar = 100 μm. Data are representative of three animals. **(C)** Sections of 130-day-old mSOD1^{G93A} mouse spinal cord were co-stained with Cy5-conjugated anti-phosphorylated JAK2 antibodies and FITC-conjugated tomato lectin. Scale bar = 100 μm. Data are representative of three animals.

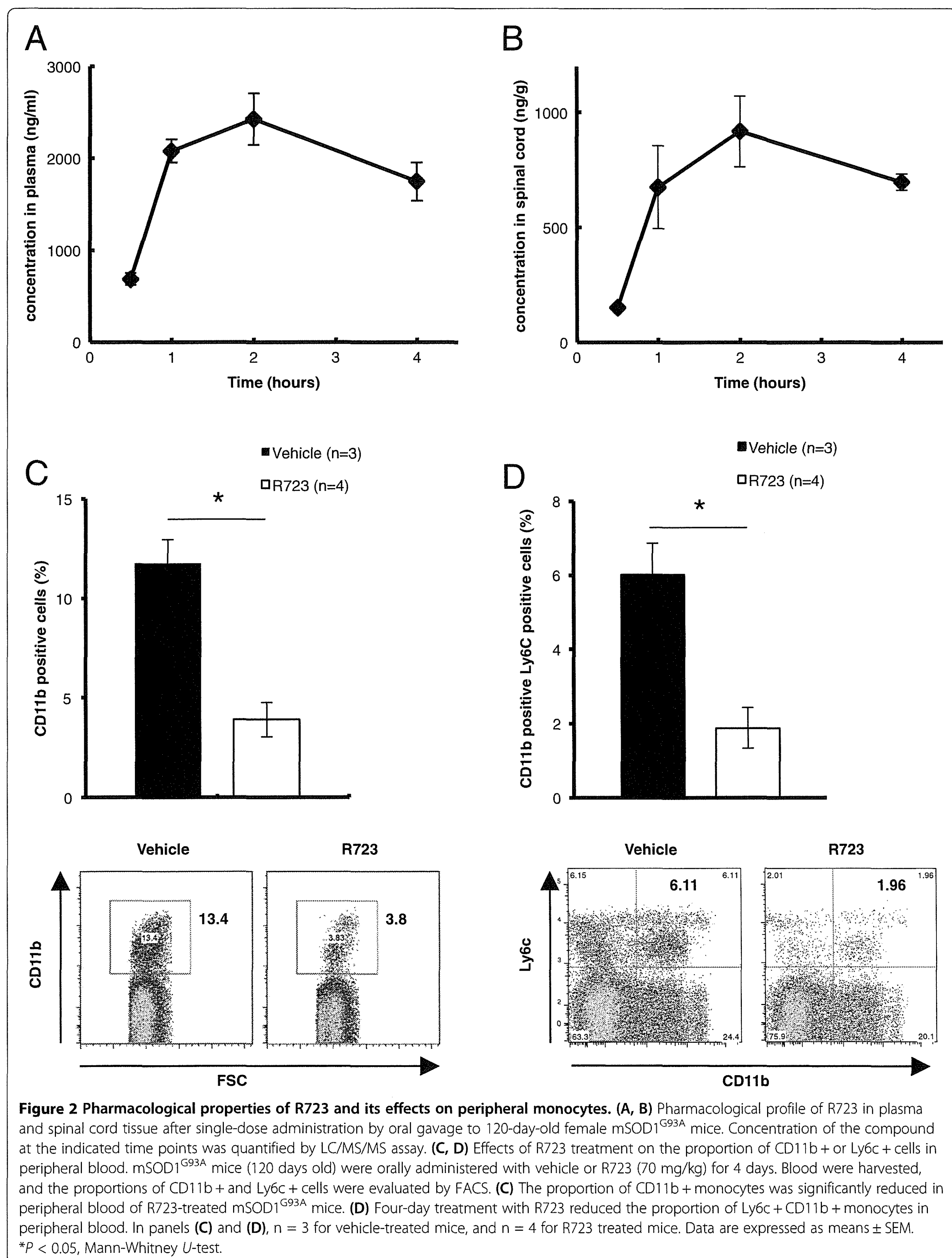

Figure 2 Pharmacological properties of R723 and its effects on peripheral monocytes. (A, B) Pharmacological profile of R723 in plasma and spinal cord tissue after single-dose administration by oral gavage to 120-day-old female mSOD1^{G93A} mice. Concentration of the compound at the indicated time points was quantified by LC/MS/MS assay. **(C, D)** Effects of R723 treatment on the proportion of CD11b + or Ly6c + cells in peripheral blood. mSOD1^{G93A} mice (120 days old) were orally administered with vehicle or R723 (70 mg/kg) for 4 days. Blood were harvested, and the proportions of CD11b + and Ly6c + cells were evaluated by FACS. **(C)** The proportion of CD11b + monocytes was significantly reduced in peripheral blood of R723-treated mSOD1^{G93A} mice. **(D)** Four-day treatment with R723 reduced the proportion of Ly6c + CD11b + monocytes in peripheral blood. In panels **(C)** and **(D)**, n = 3 for vehicle-treated mice, and n = 4 for R723 treated mice. Data are expressed as means ± SEM. *P < 0.05, Mann-Whitney U-test.

thrombocythemia and primary myelofibrosis (Additional file 2: Figure S1A) [15]. First, to investigate the drug distribution, we administered R723 by oral gavage to mSOD1^{G93A} mice and measured concentrations of R723 in serum and spinal cord tissue. R723 had sufficient access to spinal cord tissue (Figure 2A, B) (spinal area under the curve (AUC) (0.5 to 4]/plasma AUC (0.5 to 4] ratio: 0.368) [17]. Next, we tested whether R723 treatment could deplete monocytes circulating in peripheral blood. After 4 days of treatment with R723, mSOD1^{G93A} mice

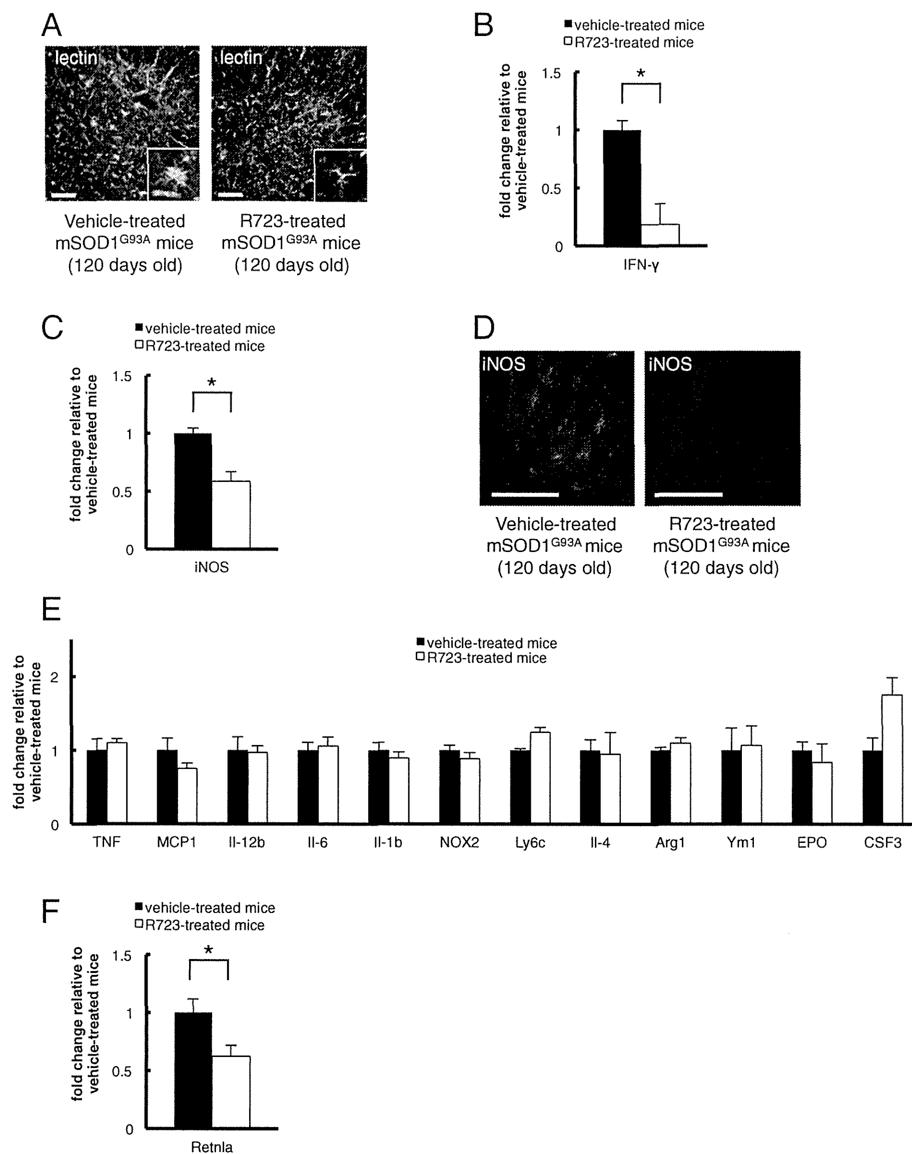

Figure 3 Effects of R723 on inflammation-related gene expression and microgliosis in spinal cord of mSOD1^{G93A} transgenic mice. (A) R723-treated mSOD1^{G93A} mice had reduced number of lectin-positive microglia in the spinal cord compared with vehicle-treated controls. Lumbar sections of the spinal cord were stained with fluorescein isothiocyanate (FITC)-conjugated tomato lectin. Scale bar = 100 μm. Data are representative of three animals. **(B, C)** Quantitative RT-PCR analyses in spinal cords of R723-treated mSOD1^{G93A} mice and vehicle-treated controls (120 days old) were performed (n = 4 in each group). The expression levels of IFN-γ and iNOS were significantly reduced in the R723-treated group (P = 0.0495 for each experiment). **(D)** Immunohistochemical analysis showed R723 treatment for 30 days had suppressed the expression level of iNOS in the spinal cords of mSOD1^{G93A} mice. Scale bar = 100 μm. Data are representative of three animals. **(E)** Quantitative RT-PCR analyses in spinal cords of R723 treated mSOD1^{G93A} mice and vehicle-treated controls (120 days old) were performed (n = 4 in each group). Relative mRNA expression is shown for TNF, MCP1, Il-12b, Il-6, Il-1b, NOX2, and Ly6c, which are related to M1 macrophages/microglia, and for Il-4, Arg1, Ym1, Il-4, EPO and CSF3, which are related to M2 macrophages/microglia. There were no significant differences in the expression levels of these molecules between two groups after the correction of multiple comparisons. **(F)** Quantitative RT-PCR analysis revealed that R723 had suppressed the expression of Retnla after 30 days of treatment in the spinal cords of mSOD1^{G93A} mice (P = 0.0495, n = 4 in each group). Data are expressed as means ± SEM. *P < 0.05, Mann-Whitney U-test.

had significantly fewer CD11b-positive cells and Ly6c-positive monocytes in peripheral blood (Figure 2C, D and Additional file 1: Supplementary information).

To further confirm the anti-inflammatory effect of R723, we evaluated the microgliosis and astrocytosis in spinal cord tissue of R723-treated mSOD1^{G93A} mice. Lectin staining revealed that R723 treatment had suppressed microgliosis in the spinal cords of mSOD1^{G93A} mice, although it did not affect astrocytosis (Figure 3A and Additional file 3: Figure S2A). In addition, we evaluated the mRNA expression of inflammation-related and M1/M2 microglia-related genes in spinal cord tissue of R723-treated mSOD1^{G93A} mice. Consistent with the anti-inflammatory effects of JAK2 inhibitor as previously reported [17], R723 treatment suppressed IFN-γ and iNOS expression dose-dependently, suggesting that the drug exerted anti-inflammatory effects in the spinal cords of mSOD1^{G93A} mice (Figure 3B, C and Additional file 4: Figure S3A). In addition, the effect of R723 against iNOS expression was confirmed by immunohistochemical analysis (Figure 3D). However, R723 had no obvious effects on other inflammatory molecules such as TNF, Il-12b, Il-6, Il-1β, and NOX2. Additionally, there was no significant difference between two groups in the spinal cord expression levels of monocyte chemotactic protein 1 (MCP1) and Ly6c, which are important for the migration and activation of inflammatory monocytes, as well as those of Il-4, arginase, liver (Arg1), chitinase-3-like 3 (Ym1), erythropoietin (EPO), and colony-stimulating factor 3 (CSF3), which are involved in the activation of M2 microglia (Figure 3E). Unexpectedly, R723 suppressed expression of resistin-like alpha (Retnla), a marker of anti-inflammatory M2 microglia, in spinal cord tissue of mSOD1^{G93A} mice after 30 days of treatment, although this effect was not evident after 5 days of treatment (Figure 3F and Additional file 4: Figure S3B). Collectively, these results suggest that oral administration of R723 decreased the number of Ly6c-positive monocytes in peripheral blood

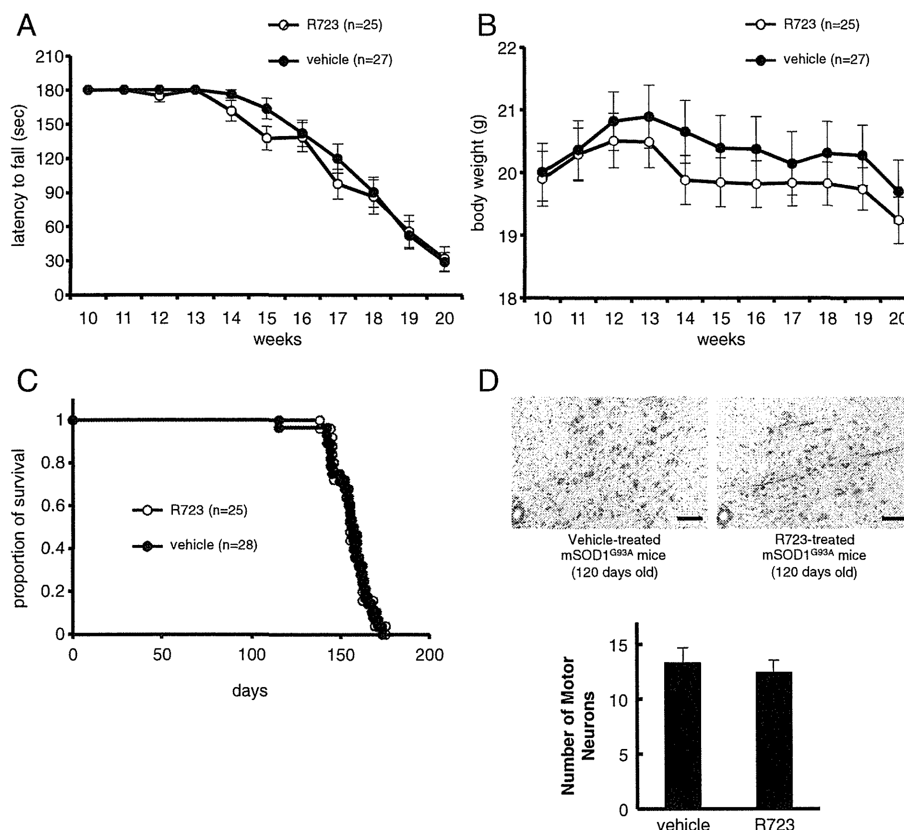

Figure 4 Body weight, motor performance, and survival of R723- or vehicle-treated mSOD1^{G93A} mice. (A) Time course of motor performance in the rotarod test. Performance of R723-treated (open circles, n = 25) and vehicle-treated (filled circles, n = 27) mSOD1^{G93A} mice were comparable. **(B)** Changes in mean body weight of R723-treated (n = 25) and vehicle-treated (n = 27) mSOD1 ^{G93A} mice. **(C)** Kaplan-Meier survival curve. R723 treatment did not affect the survival of mSOD1 ^{G93A} mice (R723-treated group, n = 25; vehicle-treated group, n = 28) ($P = 0.964$). **(D)** Nissl staining of transverse sections of lumbar spinal cord. Motor neurons were counted and statistical analysis is shown. Scale bar = 50 μm. Data are expressed as means ± SEM. Statistical analysis was performed using ANOVA **(A, B)**, log-rank test **(C)**, and the Mann-Whitney *U*-test.

and reduced the expression of several inflammatory genes in the spinal cords of mSOD1^{G93A} mice, leading to suppressed microglial activation.

Because R723 suppresses several pathways that seem to be harmful in ALS, we tested whether R723 could ameliorate neurodegeneration in mSOD1^{G93A} mice. Oral administration of R723 (70 mg/kg, twice daily; 5 days on, 2 days off) to mSOD1^{G93A} mice was started at 90 days of age and continued until 120 days of age. Motor performance was evaluated by rotarod test, and muscle atrophy was monitored by body weight reduction. Decline in motor performance of the R723-treated mSOD1^{G93A} mice was compared with that of the vehicle-treated littermates. Throughout the disease process, there was no significant change in rotarod performance or body weight between the two groups (Figure 4A, B) ($P > 0.05$ for each time point, ANOVA). Additionally, survival times for R723-treated and vehicle-treated mSOD1^{G93A} mice were comparable (Figure 4C) (average survival time; R723 treated group: 155.6 ± 1.8 days (n = 25); vehicle-treated group: 155.1 ± 2.2 days (n = 28), $P = 0.96$, log-rank test). Consistent with these observations, Nissl staining revealed that R723 treatment had led to unaltered motor neuron survival in the spinal cords of mSOD1^{G93A} mice in both groups (Figure 4D). Collectively, these results showed that R723 penetrated the spinal cord of mSOD1^{G93A} mice and suppressed inflammation, but did not affect neurodegeneration *in vivo*.

Discussion

In this study, we tried to suppress harmful inflammatory processes in mSOD1^{G93A} mice by treating them with a JAK2 inhibitor. Although R723 was effective in depleting Ly6c-positive monocytes and suppressing IFN-γ and iNOS expression in the spinal cord, the drug did not affect disease progression or survival of these mice.

We examined the expression levels of inflammation-related genes including *TNF, MCP1, Il-1β*, and *NOX2*, which play critical roles in the pathogenesis of ALS [3], and found that they were not reduced after JAK2 inhibition. It is possible that inflammation driven by these molecules masked the effects of reductions in IFN-γ and iNOS expression.

One explanation for the lack of a neuroprotective effect of R723 could be the suppression of Retnla, an M2 microglia-related gene. M2 microglial activation, which is driven by Il-4 and Il-13 produced by Th2 lymphocytes, exerts protective roles in ALS [3]. Recently, another group reported that JAK2 is activated after the recruitment of Il-13 to its receptor, and revealed that Il-13 utilizes the JAK2 signaling pathway [18]. Therefore, we speculate that suppression of Retnla counteracts the anti-inflammatory effects of R723, preventing it from exerting a neuroprotective effect *in vivo*.

Alternatively, R723 might have inhibited a neuroprotective effect of JAK2. There is a report that suggests JAK2 signaling is implicated in the prevention of neuronal apoptosis in traumatic brain injury [19].

In conclusion, R723 alone was not sufficient to protect against neurodegeneration in mSOD1^{G93A} mice, although it suppressed the expression of several proinflammatory molecules and depleted monocytes. Based on our results, it is possible that in order to ameliorate neurodegeneration in ALS, we need not only to suppress JAK2 mediated inflammation but also prevent other inflammatory pathways. Furthermore, we may need to activate neuroprotective M2 microglia to alleviate neurodegeneration in ALS.

Additional files

Additional file 1: Supplementary information. Immunohistochemical analysis of the spinal cord of mSOD1^{G93A} mice and flow cytometric analysis of the peripheral blood monocytes of mSOD1^{G93A} mice. (A) Sections of mSOD1^{G93A} mouse spinal cord were co-stained with FITC-conjugated anti-CD206 receptor antibodies and Cy5-conjugated anti-iNOS antibodies. Scale bar = 200 μm. (B) The number of Ly6c positive and CD11b positive blood monocyte remained unchanged along with the disease progression. Peripheral blood cells were collected from mSOD1^{G93A} mice (70 days old mice and 130 days old ones). The following antibodies were used: APC-Cy7-labeled anti-CD11b and FITC-labeled anti-Ly6c. Flow cytometry was performed using a FACS Canto™ II with the Diva ™ software and acquired data were analyzed using the FlowJo software.

Additional file 2: Figure S1. R723 is a selective small-molecule JAK2 inhibitor. (A) Chemical structure of R723 is shown.

Additional file 3: Figure S2. R723 had no effect on astrocytosis in the spinal cords of mSOD1^{G93A} mice. (A) The number of GFAP-positive astrocytes in the spinal cord did not differ between R723-treated mSOD1^{G93A} mice and vehicle-treated controls. Lumbar sections of the spinal cord were stained with Alexa Fluor 488®-conjugated anti-GFAP antibody. Scale bar =100 μm. Data are representative of three animals.

Additional file 4: Figure S3. R723 had a dose-dependent effect on the suppression of inflammation-related genes. (A) Quantitative RT-PCR analyses revealed that lower dose of R723 (17.5mg/kg, twice a day, 5 days on/2 days off regimen) did not change the expression profiles of IFN-γ, iNOS and Retnla in the spinal cords of mSOD1^{G93A} mice (n = 3 in lower dose group and n = 4 in other groups). (B) Quantitative RT-PCR analyses in spinal cords of R723-treated mSOD1^{G93A} mice and vehicle-treated controls were performed after 5 days of treatment (n = 3 in each group). The expression level of iNOS was significantly reduced in the R723-treated group ($P = 0.0495$). Data are expressed as means ± SEM. $^{*}P < 0.05$, Mann-Whitney *U*-test.

Abbreviations

ALS: amyotrophic lateral sclerosis; ANOVA: analysis of variance; Arg1: arginase, liver; AUC: area under the curve; CSF3: colony-stimulating factor 3; DAPI: 4',6-diamidino-2-phenylindole; EAE: experimental autoimmune encephalitis; EPO: erythropoietin; FITC: fluorescein isothiocyanate; GFAP: glial fibrillary acidic protein; GM-CSF: granulocyte macrophage-colony stimulating factor; IFN: interferon; Il: interleukin; JAK2: Janus kinase 2; JAKs: Janus kinases; MCP1: monocyte chemotactic protein 1; NOX2: NADPH oxidase 2; PBS: phosphate-buffered saline; PCR: polymerase chain reaction; RA: rheumatoid arthritis; Retnla: resistin-like alpha; TBST: tris-buffered saline with tween; TNF: tumor necrosis factor; Ym1: chitinase-3-like 3; iNOS: nitric oxide synthase 2, inducible; mSOD1^{G93A} mice: transgenic mice overexpressing the familial ALS-associated G93A SOD1 mutation.

Competing interests

YH is an employee of Rigel Pharmaceuticals, Inc. The remaining authors declare that they have no competing interests.