Note

Newly Developed Highly Bioavailable Curcumin Formulation, curcuRouge™, Reduces Neutrophil/Lymphocyte Ratio in the Elderly: A Double-Blind, Placebo-Controlled Clinical Trial

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Summary Elevated neutrophil/lymphocyte ratio (NLR) has been reported as a sensitive marker for predicting poor prognosis in chronic inflammation-based diseases such as stroke, heart failure, cancers, and diabetes, as well as acute inflammatory diseases such as bacterial and viral infections, including COVID-19. NLR is also known to increase with age and is considered to be an aging marker. We conducted a double-blind, placebo-controlled trial in elderly volunteers to examine the effect of a newly developed, highly bioavailable curcumin formulation (curcuRouge™) on NLR. Both the white blood cell count and the neutrophil rate decreased significantly, and the lymphocyte rate increased significantly from baseline to after curcuRouge™ administration for 4 wk. curcuRouge™ significantly reduced the NLR (p=0.020). On the other hand, in the placebo group, there were no changes in white blood cell count, neutrophil ratio, lymphocyte ratio, or NLR. The present study demonstrates for the first time, in elderly volunteers, that administration of curcuRouge™ significantly reduces NLR, an indicator of prognosis in cardiovascular diseases, cancer, infectious diseases, and aging. Thus, curcuRouge™ might be expected to improve the prognosis of these diseases as well as exhibit anti-aging effects.

Key Words neutrophil/lymphocyte ratio (NLR), inflammation, curcumin, curcuRouge™, anti-aging

NLR has been reported as a sensitive marker for predicting chronic inflammation-based diseases such as colorectal cancer, diabetes, stroke, and heart failure, as well as poor prognosis for infectious diseases, including coronavirus disease 2019 (COVID-19) (*1–3*). In addition, NLR is known to increase with age, and a Rotterdam study has shown that high NLR shortens lifespan in the general population over the age of 45 (*4*). For these reasons, NLR is now recognised as a disease prognostic and aging marker that is closely associated with survival or lifespan. Nevertheless, to date, there have been few attempts to lower NLR by pharmacological or dietary intervention, and there is only one report that vitamin D lowers the NLR in a study of adolescent girls around 14 y old (*5*).

Curcumin is known to exhibit antitumor, anti-inflammatory, antioxidant, and anti-amyloid activities (*6*). Curcumin is also beneficial in chronic heart failure and has been reported to suppress the progression of heart failure in a rat model with hypertensive heart disease and myocardial infarction (*7*). Highly absorptive curcumin has been shown to significantly reduce α1-antitrypsin-low density lipoprotein, one of the oxidised low-density lipoproteins, compared to a placebo in patients with mild chronic obstructive pulmonary disease (*8*). Furthermore, curcumin has been shown to suppress influenza virus growth in vitro and inflammatory cytokine production and pneumonia aggravation in an influenza virus-induced in vivo pneumonia model (*9*).

Curcumin is insoluble in water and has extremely low bioavailability. We have now developed a highly bioavailable curcumin formulation, curcuRouge™, which reaches a blood concentration 150 times higher than that of general curcumin and the pharmacokinetics of curcuRouge™ was described in our previous report (*10*). The Cmax was 493±352 ng/mL when curcuRouge™ (90 mg of curcumin) was orally administrated. We conducted a double-blind, placebo-controlled study in elderly volunteers to investigate the effect of curcuRouge™ on NLR.

Materials and Methods

Subjects. This study was conducted at the National Hospital Organization Kyoto Medical Center in Japan from June to August 2020. A total of 40 elderly volunteers aged >60 y were recruited for this study. We

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Table 1. Baseline measurements.

		n	Placebo group	*n*	curcuRouge™ group	*p*-value
Gender		20		19		0.082
	Men		17		11	
	Women		3		8	
Age (1)		20	70.0 [65.3, 74.5]	19	67.0 [65.0, 75.0]	0.955
Smoking		20		19		0.078
	Never		3		9	
	Past		15		9	
	Current		2		1	
Alcohol Drinking		16		13		0.926
	Non		6		6	
	Social		2		1	
	Occasional light		2		0	
	Occasional heavy		2		1	
	Regular		4		5	
Historys		20		19		
	Diabetis		6		9	0.333
	Cancer		5		4	>0.999
	CVD		9		9	>0.999

(1) median [IQR].

Table 2. White blood cell composition on baseline and after the administration of curcuRouge™.

	n	Baseline	After the administration	*p*-value
White blood cell count	19	6,300.0 [5,200.0, 7,800.0]	5,800 [4,800.0, 6,800.0]	0.033
Neutrophil count (/μL)	19	3,224.0 [2,909.7, 4,352.4]	2,920.2 [2,317.7, 3,651.8]	0.003
Neutrophil ratio (%)	19	56.1 [51.2, 61.4]	52.1 [47.2, 56.8]	0.004
Lymphocyte count (/μL)	19	2,167.2 [1,695.2, 2,626.0]	2,197.8 [1,724,8, 2,455.2]	0.809
Lymphocyte ratio (%)	19	32.8 [28.7, 38.1]	38.4 [31.6, 39.9]	0.038
Neutrophil/Lymphocyte ratio	19	1.70 [1.3, 2.1]	1.36 [1.2, 1.7]	0.020
Eosinophil count (/μL)	19	180.2 [132.5, 326.8]	186.0 [129.2, 347.1]	0.469
Eosinophil ratio (%)	19	3.2 [2.5, 5.5]	3.5 [2.2, 5.4]	0.809
Basophil count (/μL)	19	44.1 [29.4, 58.5]	40.8 [30.1, 46.4]	0.376
Basophil ratio (%)	19	0.7 [0.6, 0.8]	0.7 [0.6, 0.7]	0.561

data: median [IQR].

recruited healthy elderly volunteers (≥60 y old, no gender restrictions) through posters or our website and invited the general public to participate in the study after providing voluntary written consent. In the case of applicants with lifestyle-related diseases who were undergoing medical treatment, it was possible to enroll them after confirming that their condition was stable. The exclusion criteria were as follows: 1) regular consumption of foods containing curcumin; 2) history of allergy to curcumin; 3) pregnancy or breastfeeding; 4) receipt of treatment for malignant tumors; 5) regular use of antibiotics or steroids; 6) use of two or more anti-platelet agents or one anti-platelet agent and another anti-thrombotic agent (anti-coagulant, EPA agent, prostacyclin agent); 7) history of cerebral hemorrhage and current use of an anti-platelet agent; 8) use of home oxygen therapy; 9) dialysis for renal failure; 10) serious liver dysfunction or cirrhosis; 11) severe cardiac dysfunction (left ventricular ejection fraction <20%); and 12) judged to be unsuitable for participation in this study by the principal investigators and sub-researchers. All subjects provided written informed consent to participate in a double-blind, placebo-controlled trial of curcuRouge™ approved by the Kyoto Medical Center Ethics Review Board. The trial was registered with the UMIN Clinical Trials Registry (9 July 2020 UMIN 000041042).

Study design. At baseline, blood samples were collected to obtain blood data. Subjects were double-blindly randomised into two groups: curcuRouge™ (administered 90 mg/capsule curcumin) and placebo (administered a replacement of cornflour instead of curcumin). In both groups of subjects, one capsule was taken each time, twice daily in the morning and evening, and a blood sample was taken 4 wk later. After the completion of oral administration, the subject was queried to deter-

Table 3. White blood cell composition on baseline and after the administration of placebo.

	n	Baseline	After the administration	*p*-value
White blood cell count	20	5,900.0 [4,850.0, 6,500.0]	5,800.0 [4,825.0, 6,750.0]	0.158
Neutrophil count (/μL)	20	3,159.2 [2,245.2, 3,798.6]	2,919.2 [2,156.2, 3,747.4]	0.478
Neutrophil ratio (%)	20	54.0 [42.9, 59.1]	52.2 [41.5, 61.4]	0.654
Lymphocyte count (/μL)	20	1,943.0 [1,783.1, 2,325.6]	1,914.8 [1,700.2, 2,366.4]	0.455
Lymphocyte ratio (%)	20	34.7 [30.4, 46.0]	36.6 [29.4, 45.3]	0.926
Neutrophil/Lymphocyte ratio	20	1.53[0.9, 2.0]	1.41 [0.9, 2.1]	0.881
Eosinophil count (/μL)	20	191.2 [124.5, 368.4]	167.1 [133.5, 357.8]	0.455
Eosinophil ratio (%)	20	3.4 [2.6, 6.1]	3.0 [2.3, 6.4]	0.455
Basophil count (/μL)	20	35.7 [27.0, 46.7]	33.5 [28.9, 39.7]	0.732
Basophil ratio (%)	20	0.6 [0.5, 0.8]	0.6 [0.5, 0.7]	0.557

data: median [IQR].

mine the number of remaining capsules of the test substance to confirm the dose status. An adherence rate of ≥80% was considered good. The number of subjects was based on a double-blind, parallel-group study of herbal supplement B (*11*). For blood data, NLR, neutrophil count, lymphocyte count, eosinophil count, and basophil count were measured. Placebo and curcuRouge™ capsules were generated by Therabiopharma Inc. (Kawasaki, Japan).

Statistical analyses. An unpaired t test and Mann Whitney *U* test were applied for continuous data with normal and skewed distributions, respectively. A paired *t* test was used for intragroup comparison of normally distributed data, whereas the skewed data were compared using the Wilcoxon signed-rank test. A *p* value of <0.05 was considered significant.

Results

A total of 40 volunteers aged 65 to 75 y participated in this study. One volunteer in the curcuRouge™ group was excluded from the analysis due to lack of blood sampling data after the administration. At baseline, there were no differences in age, gender distribution; smoking and alcohol consumption habits; and the history of diabetes, cancer, and cardiovascular disease (CVD) between the placebo and curcuRouge™ groups (Table 1). NLR was also similar ($p=0.261$) between the groups. Good adherence to test food intake was observed in all subjects. As shown in Table 2, at 4 wk after the administration of curcuRouge™, white blood cell count, neutrophil count, and neutrophil ratio (%) significantly decreased, and the lymphocyte ratio (%) significantly increased from the baseline, thus resulting in a significant decrease in the NLR of 0.34 ($p=0.020$). On the other hand, in the placebo group, there were no changes from baseline to after the administration in white blood cell count, neutrophil count, neutrophil ratio, lymphocyte count, lymphocyte ratio, and NLR (Table 3). The rate of change in NLR before and after administration was −1.1% in the placebo group and −11.3% in the curcuRouge™ group. No adverse events were observed in either the curcuRouge™ or placebo groups.

Discussion

In recent years, NLR has been reported as a sensitive marker for the prognosis of cardiovascular disease, cancer, and infectious diseases such as COVID-19 (*1–3*). NLR is known to increase with age as well (*4*). In a double-blind placebo-controlled clinical trial, this study demonstrated in elderly volunteers that taking curcuRouge™ significantly reduced NLR without any safety issues. Curcumin is known to suppress chronic inflammation by inhibiting the activation of nuclear factor-kappa B (NF-κB). In colitis models, curcumin has been reported to produce therapeutic effects through its anti-inflammatory effects mediated by inhibiting nuclear factor-erythroid 2-related factor 2 (Nrf2) activation and signal transducer and activator of transcription 3 (Stat3) (*12*). It is conceivable that these anti-inflammatory mechanisms of curcumin may improve NLR. Furthermore, in this study, neutrophil counts were significantly reduced by curcuRouge™. When neutrophils are stimulated by inflammation, they activate NF-κB signaling and Janus kinases (JAK)/STAT signaling via various cytokine receptors on their surface, such as Toll-like receptor 4 (TLR4) and tumor necrosis factor α (TNFα) receptors, resulting in cytokine production, and immune cell activation. In persistent inflammation, neutrophil apoptosis is inhibited by JAK/STAT and TNFα receptor 1 (*13*). These mechanisms may lead to increases in neutrophil counts and NLR in chronic inflammation. It has been reported that curcumin inhibits the activation of JAK/STAT and NF-κB signaling, promoting neutrophil apoptosis, suppressing the sustained inflammatory response by neutrophils (*14*). Thus, curcuRouge™ might exert to improve NLR by its anti-inflammatory effects. Therefore, it might be possible that taking curcuRouge™ leads to the prevention of various age-related diseases. Further studies are necessary to increase the number of cases in elderly volunteers to prove such possibilities. High NLR is also closely associated with the development of critical illness in COVID-19 patients (*1*). The severity of pneumonia and severe thrombosis in patients with COVID-19 is believed to be caused by excessive activation of the immune system, called the cytokine storm. It has also been sug-

gested that curcumin suppresses the cytokine storm mainly by inhibiting NF-κB activation (*15*, *16*). Therefore, curcuRouge™ could be expected to suppress aggravation in patients with COVID-19. However, further studies are needed to confirm this hypothesis.

Authorship

Research conception and design: AK, TH and KH; clinical trial: KH; statistical analysis of the data: AK, HW, NSA and HY; interpretation of the data: AK, AI, TH and KH; writing of the manuscript: AK, AI and KH.

Disclosure of state of COI

Robertet Group (France) supported Therabiopharma Inc. for this work. Therabiopharma is a company that develops and markets curcuRouge™. An agreement on joint research in relation to this trial was conducted between Therabiopharma and the Kyoto Medical Center. The tested samples of curcuRouge™ and placebo were provided by Therabiopharma. The authors report no other conflicts of interest in this work.

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