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
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-
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 curcuRougeTM 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 curcuRougeTM 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 curcuRougeTM 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 curcuRougeTM, 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 curcuRougeTM group. No adverse events were observed in either the curcuRougeTM 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 curcuRougeTM significantly reduced NLR without any safety issues. Curcumin is known to suppress chronic inflammation by inhibiting the activation of nuclear factor-kappa B (NF-kB). 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 curcuRougeTM. When neutrophils are stimulated by inflammation, they activate NF-kB 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 alpha (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-kB signaling, promoting neutrophil apoptosis, suppressing the sustained inflammatory response by neutrophils (14). Thus, curcuRougeTM might exert to improve NLR by its anti-inflammatory effects. Therefore, it might be possible that taking curcuRougeTM 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-

| Table 3. White blood cell composition on baseline and after the administration of placebo. |
|-----------------------------------------------|-----------------------------------------------|
| White blood cell count | n | Baseline | After the administration | p-value |
|------------------------|---|-----------|--------------------------|---------|
| Neutrophil count (/μL) | 20 | 5,900.0 [4,850.0, 6,500.0] | 5,800.0 [4,825.0, 6,750.0] | 0.158 |
| Neutrophil ratio (%) | 20 | 3.159 [2.245.2, 3.798.6] | 2.919 [2.156.2, 3.747.4] | 0.478 |
| Lymphocyte count (/μL) | 20 | 54.0 [42.9, 59.1] | 52.2 [41.5, 61.4] | 0.654 |
| Lymphocyte ratio (%) | 20 | 3.4 [2.6, 4.6] | 3.0 [2.3, 6.4] | 0.455 |
| Basophil count (/μL) | 20 | 191.2 [124.5, 368.4] | 167.1 [133.5, 357.8] | 0.455 |
| Basophil ratio (%) | 20 | 3.4 [2.6, 6.1] | 3.0 [2.3, 6.4] | 0.455 |
| Eosinophil count (/μL) | 20 | 35.7 [27.0, 46.7] | 33.5 [28.9, 39.7] | 0.732 |
| Eosinophil ratio (%) | 20 | 0.6 [0.5, 0.8] | 0.6 [0.5, 0.7] | 0.557 |

Data: median [IQR].
gested that curcumin suppresses the cytokine storm mainly by inhibiting NF-κB activation (15, 16). Therefore, curcuRouge™ could be expected to suppress aggrava- tion 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 and KH; writing of the manuscript: AK, AI and KH.

**Disclosure of state of COI**

Robertert 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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