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Curcumin Inhibits WEHI-3 Leukemia Cells in BALB/c Mice *In Vivo*

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Abstract. *Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5- dione), a natural polyphenol product of the plant Curcuma longa, exhibited potent inhibitory activities against proliferation, induced cell cycle arrest and exhibited the induction of apoptosis in several tumor cell lines. In our previous studies, we have shown that curcumin induced cell cycle arrest and apoptosis on human leukemia HL-60 and mouse leukemia WEHI-3 cells; there are no reports regarding whether or not it affects leukemia cells in vivo. In the present study, we investigated the effects of curcumin on WEHI-3 in BALB/c mice and the results indicated that curcumin reduces the percentage of Mac-3 marker, which is the precursor of macrophage. Curcumin induced significant effects on the population of B cells from murine leukemia in vivo. We also investigated the weights of spleen and liver from murine leukemia and the results showed that curcumin reduced the weight of the liver and spleen. From the pathological examinations, the effects of curcumin on the liver and spleen from mice after being injected with WEHI-3 cells were apparent. Both organs were enlarged. In conclusion, curcumin affect WEHI-3 cells in vivo.*

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6.heptadiene-3,5-dione), is a natural product and the active portion of turmeric (*Curcuma longa L*), and has been shown to have biological activities, such as antioxidant (1-3), anti-inflammatory, anticarcinogenic, antiviral, hypolipidemic, and anti-infectious activities (3-5). In particular, many reports have demonstrated that curcumin induces apoptosis in human cancer cell lines, such as HL-60, K562, MCF-7, HeLa (6) and HT29 (7). In our earlier studies, we have also found that curcumin inhibited *N*-acetyltransferase activity and gene expression (8) and induced apoptosis *via* reactive oxygen species (ROS) in human colon cancer colo 205 cells (9). Recently, we also found that curcumin induced cell cycle arrest and apoptosis in human acute promyelocytic leukemia HL-60 cells through a mitochondria-dependent pathway and the activation of caspase-3 (10). Interestingly, curcumin induced apoptosis in scleroderma lung fibroblasts (SLF) without affecting normal lung fibroblasts (NLF) (11). Although many reports showed that curcumin affected many human and mouse cancer cell lines even WEHI-3 cells, there is no available information to address whether curcumin affects the leukemia cells *in vivo*. Hence, in the present study, we investigated the *in vivo* effect of curcumin on the mouse leukemia WEHI-3.

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Materials and Methods

Materials and reagents. Curcumin and olive oil were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640, fetal bovine serum, penicillin-streptomycin and glutamine were obtained from Gibco BRL (Grand Island, NY, USA).

Male BALB/c mice. Sixty male BALB/c mice, weighing 22-28 g (8 weeks old), were obtained from the Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei, Taiwan, ROC).

Murine WEHI-3 leukemia cells. The murine myelomonocytic leukemia WEHI-3 cells were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan, ROC). The cells were placed into 75-cm^3 tissue culture flasks in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin) and 1% glutamine and grown at 37˚C under a humidified 5% CO_2 atmosphere.

In vivo studies

i.) Curcumin treatment. The BALB/c mice were randomly divided into 6 groups receiving different treatments. These experiments were divided into two parts:

Part I. Normal animals (30 BALB/c mice) were divided into 3 groups of 10 animals. Group I formed the control; group II was treated with PBS (vehicle); group III was treated with curcumin in PBS.

Part II. Normal animals (30 BALB/c mice) were divided into 3 groups of 10 animals. Group I was injected with WEHI-3 cells only as control; group II was injected with WEHI-3 cells, and was then treated with curcumin (30 μg/100 μL) in PBS; group III was injected with WEHI-3 cells and then was treated with curcumin (60 μM/100 μL) in PBS. All animals were given the above dose *per os* daily before for up to 3 weeks being weighed (12, 13). Curcumin was dissolved in PBS (Sigma, MO, USA) to treat mice.

ii) Blood samples and immunofluorescence staining. Blood was collected (about 1 mL) from each animal of each group at the end of the experiments and was immediately treated with ammonium chloride for lysing of the red blood cells, followed by centrifugation for 15 minutes at 1500 rpm at 4˚C (12, 13). The isolated white blood cells were examined for cell markers (Mac-3, CD11b, CD3, CD14 and CD19) based on staining with anti-Mac-3, -CD11b, -CD3, -CD14 and -CD19 antibodies (Pharminge, San Diego, CA, USA). These cells were then stained with the second fluorescent antibody before being analyzed to determine the cell marker levels by flow cytometry (FACS Calibur TM, Becton Dickinson, NJ, USA) as described elsewhere (12-14).

iii) Tissue samples (liver and spleen). Each animal from each group was weighed before blood was collected and sampled. The liver and spleen samples were obtained, weighed individually and used for histopathology.

iv) Histopathology. Isolated spleen and liver were fixed in 4% formaldehyde and embedded in paraffin. Sections of 5 mm were stained with hematoxylin and eosin according to standard procedures (12, 13).

v) Statistics. The results were expressed as mean±SD and the difference between groups was analyzed by one-way ANOVA. $^*P<0.05$ was considered significant.

Results

WEHI-3 cells induced leukemia tumors in animals. Figure 1 shows the presence of leukemia tumors in animals 3 weeks after injection with WEHI-3 cells. Histopathologically, neoplastic cells mainly infiltrated the sinusoid and the portal tract of the liver; some neoplastic cells also destroyed the hepatic cord and tumor embolism was observed. The spleens showed marked expansion of red pulp but the white pulp indicated little change. The neoplastic cells contained large irregular nuclei with clumped chromatin, prominent nucleoli and abundant clear and light eosinophilic cytoplasm.

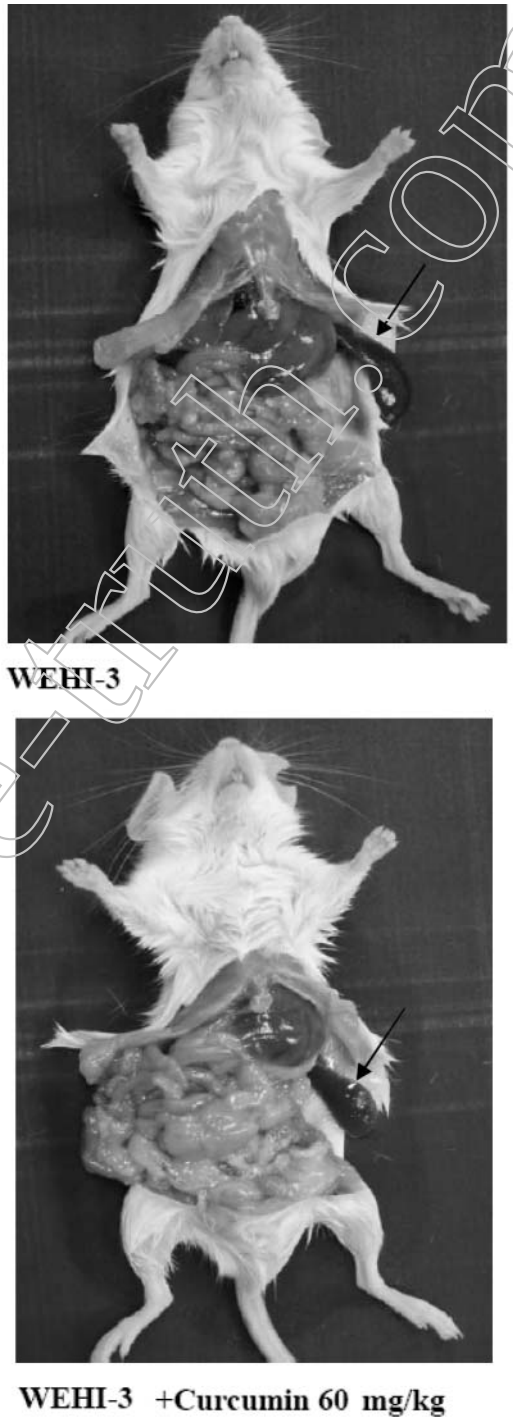

Figure 1. *Representative images of BALB/c mice after injection with WEHI-3 cells for 3 weeks. After injection with WEHI-3 cells (1x10^6 cells/100 μL) in PBS for 3 weeks, blood was collected and animals were sacrificed, and then photographed. The arrow point is size of spleen.*

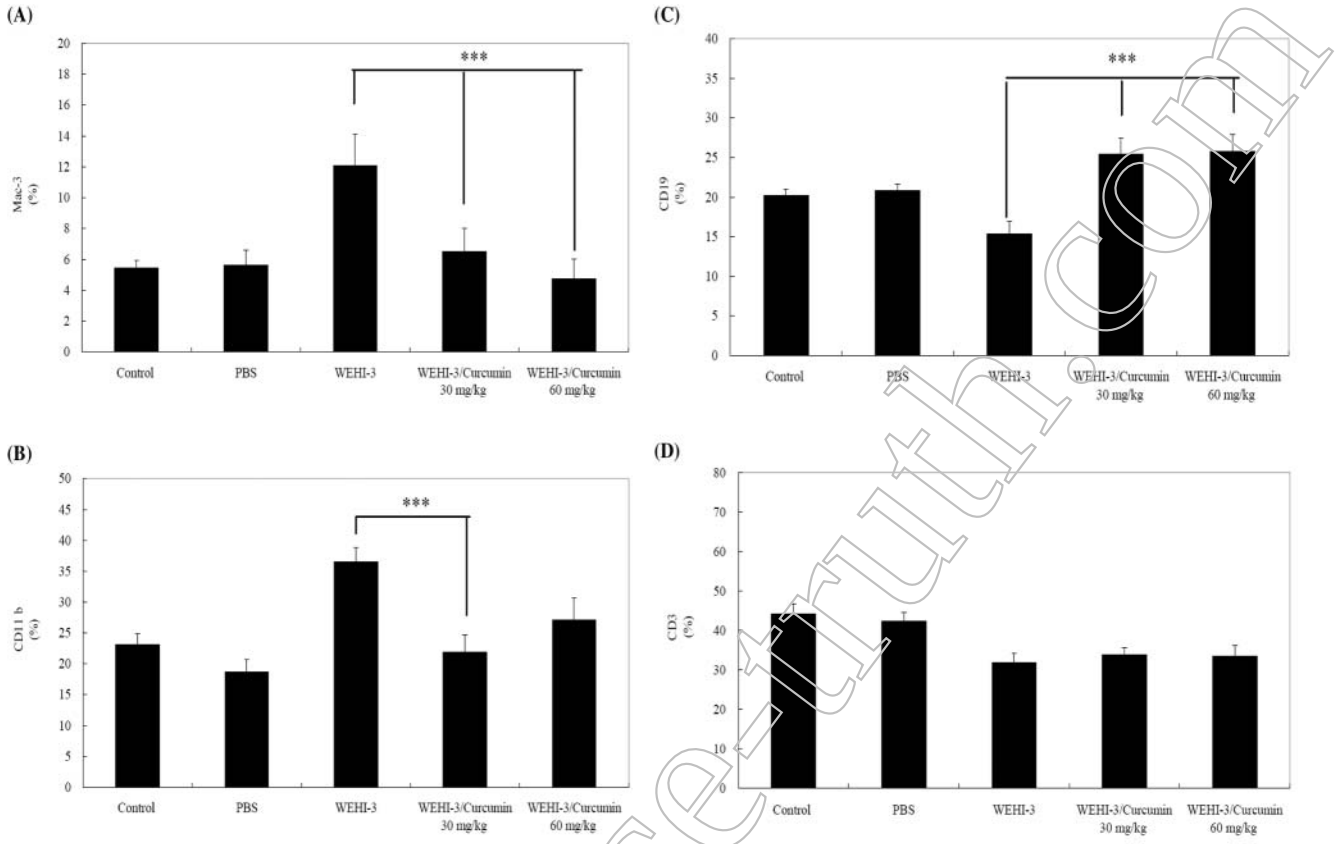

Figure 2. *Curcumin affected the white blood cell surface markers after injection with WEHI-3 cells in BALB/c mice. The animals were injected with WEHI-3 cells (1x10^6 cells/100 μL) in PBS for 3 weeks then treated with or without curcumin for 3 weeks. Blood was collected and analyzed for cell markers by flow cytometry as described in Materials and Methods. Each point is the mean±S.D. of three experiments. ***P<0.05.*

Curcumin affected the white blood cell surface markers after injection of WEHI-3 cells in BALB/c mice. The data for cell markers of white blood cells from BALB/c mice after treatment with or without curcumin in PBS are presented in Figure 2. Curcumin induced a significant difference in Mac-3 and CD19 between WEHI-3 only treated groups ($p<0.05$).

Curcumin affected the morphological size, weight and histopathology of the liver. Each liver was isolated from all animals and photographed, weighed and histopathologically examined. Representative results, presented in Figure 3A, B and C, show that curcumin affected the morphology, weight and cells of the liver. The liver demonstrated a pattern ranging from minimal histopathological change to scanty small neoplastic cell nests present in the sinusoid which exhibited apoptosis.

Curcumin affected the morphological size, weight and histopathology of the spleen. Each spleen was isolated from all animals and were photographed, weighed and histopathologically examined. The representative results are presented in Figure 4A, B and C showing that curcumin affected the morphology, weight and cells of liver. Spleen samples demonstrated markedly decreased numbers of neoplastic cells or the cells were hard to find in the red pulp. Moreover, the number of megakaryocytes increased.

Discussion

In our previous studies, curcumin was shown to inhibit the proliferation of HL-60 (10) and colon cancer colo 205 (9) cells and induce apoptosis through mitochondria-dependent and caspase-3 pathways (9, 10), raising the possibility that curcumin may have some chemotherapeutic value for human leukemia (10, 11). However, the effects of curcumin on leukemia *in vivo* provide no clear information. Our earlier studies have also shown that curcumin induced cytotoxicity in HL-60 cells. We also found that curcumin induced cytotoxicity in WEHI-3 cells (data not shown).

The *in vivo* model through the mice injected *i.p.* with WEHI-3 is well established (15) and has also been demonstrated in our previous studies (12, 13). Murine monomyelocytic

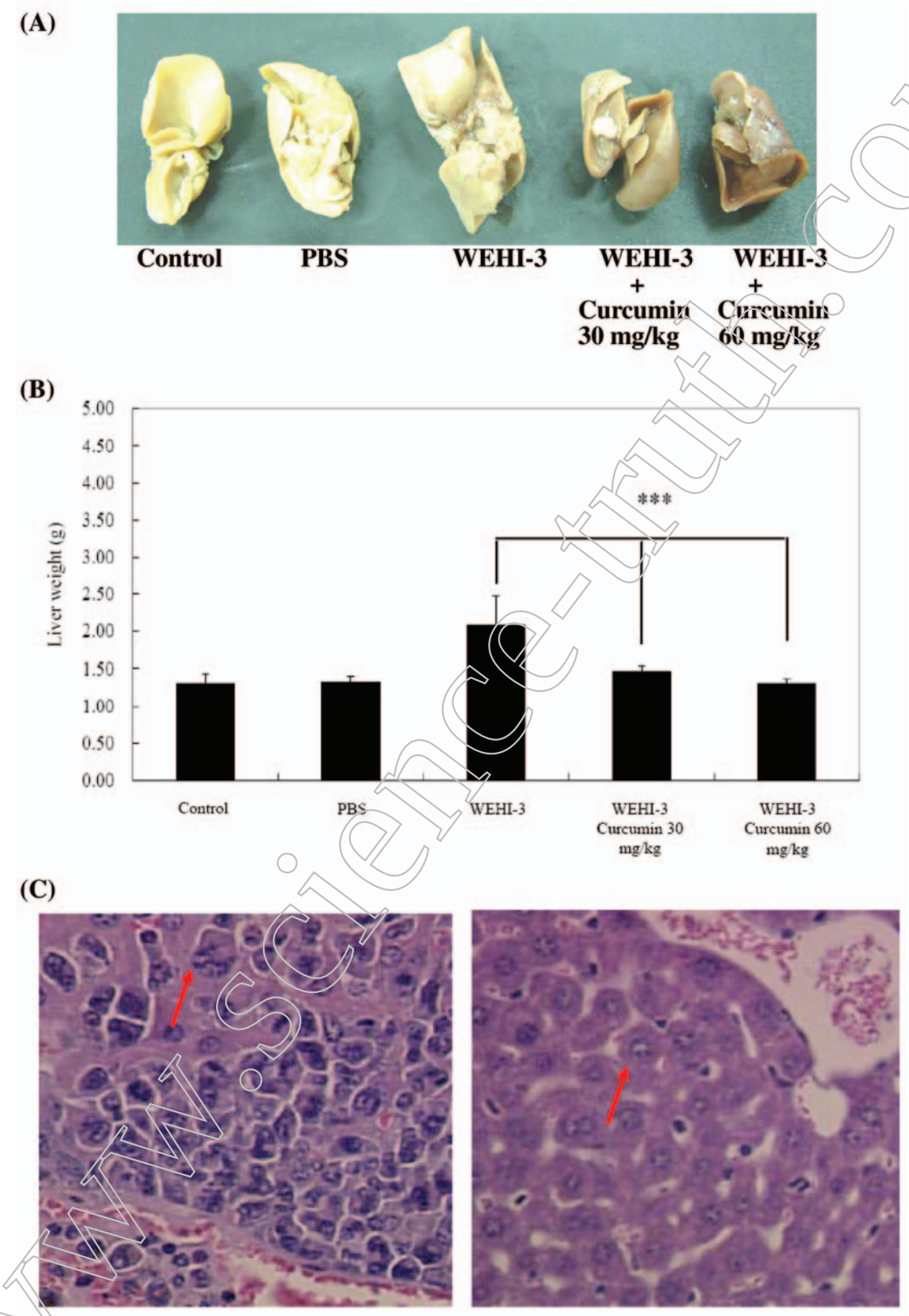

Figure 3. *Curcumin affected the morphological size, weight and histopathology of liver. Livers were photographed (A), weighed (B) and histopathologically examined (C).*

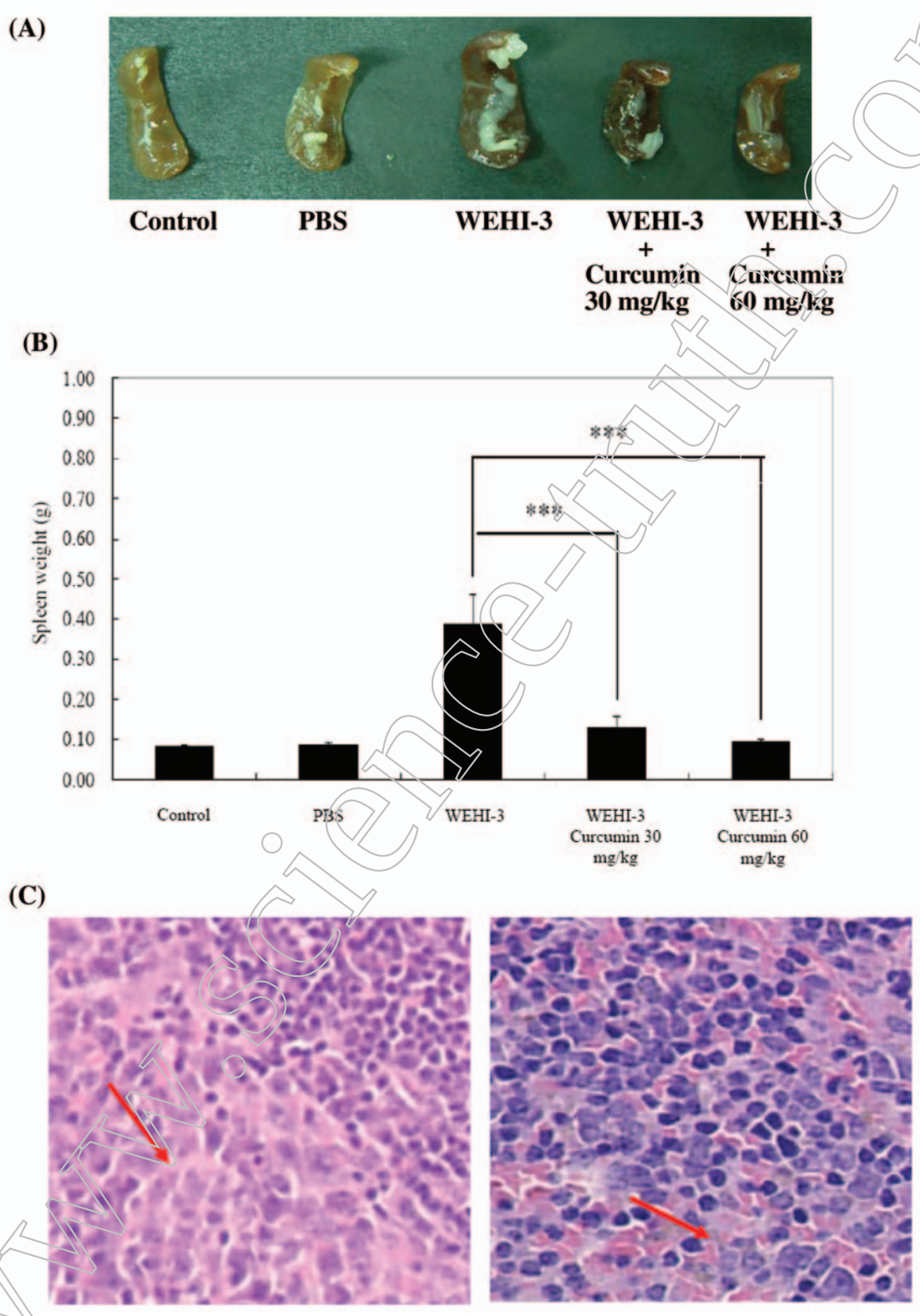

Figure 4. *Curcumin affected the morphological size, weight and histopathology of spleen. Spleens were photographed (A), weighed (B) and histopathologically examined (C).*

WEHI-3 leukemia cells were originally derived from the BALB/c mouse (16) and have been demonstrated to be an ideal system for the study of potential therapeutic drugs (ATRA, aclacinomycin A, IL-6, G-CSF and vitamin D3) which could induce *in vitro* differentiation of WEHI-3 cells in monocytic and granulocytic lineages (17-21) and the number of potential growth-regulatory secretory products from this cell line is recognized to be very large.

Therefore, the purpose of this study was to examine the effects of curcumin on WEHI-3 tumor cells in BLAB/c mice *in vivo*. At first, we examined curcumin in normal control animals after 16 weeks treatment with curcumin at 30 and 60 μM and examination of the sacrificed animals did not reveal abnormalities at the examined dose (data not shown). Based on these observations, we subsequently injected WEHI-3 cell *i.p.*, followed by orogastoric tube for curcumin treatment before sacrificing animals for examination. The results from these experiments demonstrated that curcumin statistically reduced the percentage of Mac-3 cells in the blood.

In the present report, we showed that curcumin inhibited spleen leukemia tumor growth in a WEHI-3 leukemia murine model. A notable characteristic of this model is the elevation of peripheral monocytes and granulocytes with immature morphology and apparently enlarged and infiltrated spleens as compared with normal counterpart (15). However, in our experiments we observed that in the curcumin-treated groups, the size of the spleen decreased and there was a significant difference between the control and curcumin-treated groups. These observations were also seen in liver tissues. Thus, the influence of curcumin on apoptosis induction, already the subject of many studies, as well as an understanding of its mechanism of action *in vivo,* should be further investigated in terms of signal pathway factors.

Acknowledgements

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References

1 Sharma OP: Antioxidant activity of curcumin and related compounds. Biochem Pharmacol *25*: 1811-1812, 1976.

2 Joe B, Vijaykumar M and Lokesh BR: Biological properties of curcumin-cellular and molecular mechanisms of action. Crit Rev Food Sci Nutr *44*: 97-111, 2004.

3 Krishnaswamy K, Goud VK, Sesikeran B, Mukundan MA and Krishna TP: Retardation of experimental tumorigenesis and reduction in DNA adducts by turmeric and curcumin. Nutr Cancer *30*: 163-166, 1998.

4 Aggarwal BB, Kumar A and Bharti AC: Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res *23*: 363-398, 2003.

5 Araujo CC and Leon LL: Biological activities of *Curcuma longa* L. Mem Inst Oswaldo Cruz *96*: 723-728, 2001.

6 Roy M, Chakraborty S, Siddiqi M and Bhattacharya RK: Induction of apoptosis in tumor cells by natural phenolic compounds, Asian Pac. J Cancer Prev *3*: 61-67, 2002.

7 Goel A, Boland CR and Chauhan DP: Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. Cancer Lett *172(2)*: 111-118, 2001.

8 Tourkina E, Gooz P, Oates JC, Ludwicka-Bradley A, Silver RM and Hoffman S: Curcumin-induced apoptosis in scleroderma lung fibroblasts: role of protein kinase cepsilon. Am J Respir Cell Mol Biol *31*: 28-35, 2004.

9 Su CC, Lin JG, Li TM, Chung JG, Yang JS, Ip SW, Lin WC and Chen GW: Curcumin-induced apoptosis of human colon cancer colo 205 cells through the production of ROS, Ca^{2+} and the activation of caspase-3.Anticancer Res *26(6B)*: 4379-4390, 2006.

10 Tan TW, Tsai HR, Lu HF, Lin HL, Tsou MF, Lin YT, Tsai HY, Chen YF and Chung JG: Curcumin-induced cell cycle arrest and apoptosis in human acute promyelocytic leukemia HL-60 cells *via* MMP changes and caspase-3 activation. Anticancer Res *26(6B)*: 4361-4372, 2006.

11 Chen JC, Hwang JM, Chen GW, Tsou MF, Hsia TC and Chung JG: Curcumin decreases the DNA adduct formation, arylamines N-acetyltransferase activity and gene expression in human colon tumor cells (colo 205). In Vivo *17(3)*: 301-309, 2003.

12 Yang JS, Kok LF, Lin YH, Kuo TC, Yang JL, Lin CC, Chen GW, Huang WW, Ho HC and Chung JG: Diallyl disulfide inhibits WEHI-3 leukemia cells *in vivo*. Anticancer Res *26(1A)*: 219-226, 2006.

13 Yu FS, Yang JS, Lin HJ, Yu CS, Tan TW, Lin YT, Lin CC, Lu HF and Chung JG: Berberine inhibits WEHI-3 leukemia cells *in vivo*. In Vivo *21(2)*: 407-412, 2007.

14 Ormerod MC: Flow Cytomtery. Oxford University Press 61-82, 2000.

15 He Q and Na XD: The effects and mechanisms of a novel 2-aminosteroid on murine WEHI-3B leukemia cells *in vitro* and *in vivo*. Leukemia Res *25*: 455-461, 2001.

16 Warner NL, Moore MAS and Metcalf A: A transplantable myelomonocytic leukemia in BALB/c mice: cytology, karyotype, and muramidase content. J Natl Cancer Inst *43*: 963-982, 1969.

17 Gamba-Vitalo C, Blair OC, Keys SR and Sartorelui AC: Differentiation of WEHI-3B D+ monomyelocytic leukemia cells by retinoic acid aclacinomycin A. Cancer Res *46*: 1189-1194, 1986.

18 Burgess AW and Metcalf D: Characterization of a serum factor stimulating the differentiation of myelomonocytic leukemia cells. Intl J Cancer *26*: 647-654, 1980.

19 Metcalf D: Actions and interactions of G-CSF, LIF, and IL-6 on normal and leukemic murine cells. Leukemia *3*: 349-354, 1989.

20 Li JM and Sartorelli AC: Synergistic induction of the differentiation of WEHI-3B D+ myelomonocytic leukemia cells by retinoic acid and granulocyte colony-stimulating factor. Leukemia Res *16*: 571-576, 1992.

21 Li JM, Finch RA and Sartorelli AC: Role of vitamin D3 receptor in the synergistic differentiation of WEHI-3B leukemia cells by vitamin D3 and retinoic acid. Exp Cell Res *249*: 279-290, 1999.

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