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Curcumin Inhibits Human Lung Large Cell Carcinoma Cancer Tumour Growth in a Murine Xenograft Model

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Curcumin can decrease viable cells through the induction of apoptosis in human lung cancer NCI-H460 cells *in vitro*. However, there are no reports that curcumin can inhibit cancer cells *in vivo*. In this study, NCI-H460 lung tumour cells were implanted directly into nude mice and divided randomly into four groups to be treated with vehicle, curcumin (30 mg/kg of body weight), curcumin (45 mg/kg of body weight) and doxorubicin (8 mg/kg of body weight). Each agent was injected once every 4 days intraperitoneally (i.p.), with treatment starting 4 weeks after inoculation with the NCI-H460 cells. Treatment with 30 mg/kg and 45 mg/kg of curcumin or with 8 mg/kg of doxorubicin resulted in a reduction in tumour incidence, size and weight compared with the control group. The findings indicate that curcumin can inhibit tumour growth in a NCI-H460 xenograft animal model *in vivo*.

Keywords: curcumin; human lung NCI-H460 cancer cells; xenograft transplantation; *in vivo*.

INTRODUCTION

In Taiwan, about 32.8 and 32.5 persons per 100 thousand die annually from lung and liver cancer respectively, based on reports from the People's Health Bureau of Taiwan in year 2006 (Department of Health, Executive Yuan, R.O.C. (Taiwan) Taipei; http://www.doh.gov.tw/EN2006/index_EN.aspx.). Currently, the treatment of these cancers involves radiotherapy, chemotherapy, or a combination of both, but mortality in both types of cancer patient remains high. Many studies have shown that certain phytochemicals can act as chemopreventive or chemotherapeutic agents in human cancer and many prescription drugs in use for cancer treatment are derived from plants (Craig, 1997; Kucuk, 2002).

Curcumin (diferuloylmethane), a phenolic compound obtained from turmeric, the rhizome of *Curcuma longa* (L.), is commonly used in food (Huang *et al.*, 1998). It has been reported that curcumin inhibits cell proliferation, induces apoptosis and has antitumour activity in many human cancer cell lines, including non-small cell lung cancer cells (Kawamori *et al.*, 1999; Rao *et al.*, 1995; Verma *et al.*, 1997). Curcumin also exhibits anticancer activities *in vitro* and *in vivo* in leukemia WEHI-3 cells (Gajate *et al.*, 2003; Su *et al.*, 2008) and has been shown to act by regulating a variety of antitumour signalling pathways (Kuttan *et al.*, 2007; Lin, 2007). It has been suggested that curcumin acts as an oral cancer preventative agent (Sharma *et al.*, 2004) although few *in vivo* studies have yet been reported. The present study focused on the anticancer effect of curcumin *in vivo* in mice, using a human lung cancer xenograft model of NCI-H460 cells.

MATERIALS AND METHODS

Chemicals. Curcumin and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical (St Louis, MO, USA).

Cell culture. Human lung large cell carcinoma cancer NCI-H460 cells were obtained from the Food Industry

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Research and Development Institute (Hsinchu, Taiwan), and maintained at 37 ºC in a humidified 5% CO_2 and 95% air in RPMI-1640 medium (Gibco-BRL, Grand Island, NY, USA) supplemented with 10% FBS (Hyclone Laboratories, Logan, UT, USA), 1% penicillin–streptomycin (100 units/mL penicillin and 100 μg/mL streptomycin) and 2 mM L-glutamine.

***In vivo* NCI-H460 tumour xenograft model.** Female athymic nude (BALB/$c^{nu/nu}$ mice) were obtained from Laboratory Animal Center of National Applied Research Laboratories (Taipei, Taiwan). All animals were maintained in standard vinyl cages with air filter tops in a filtered laminar air flow room at 25 ºC on a 12 h light/dark cycle; water and food were autoclaved and provided. The experimental design for this study is shown in Fig. 1.

NCI-H460 cells (1×10^7) in RPMI-1640 medium were injected subcutaneously into the flanks of mice. Tumour-bearing mice were then divided randomly into treatment groups (six mice per group) and treatment initiated when the xenografted solid tumours reached a volume of about 100 mm^3. Each mouse was injected i.p. every 3 days with either 30 μL of control vehicle (DMSO), curcumin (30 and 45 mg/kg) or doxorubicin (8 mg/kg). All experiments were conducted according to institutional guidelines and approved by the Animal Care and Use Committee of the China Medical University, Taichung, Taiwan. The doses of curcumin (30 and 45 mg/kg) used here are close to those used in other reports, for example human PC-3 prostate cancer (Khor *et al.*, 2006) and pancreatic cancer (Kunnumakkara *et al.*, 2007) xenografts in immunodeficient mice.

After xenograft transplantation, mice exhibiting tumours were monitored and tumour size was measured once every 3 days using calipers. The tumour volume in each animal was estimated according to the formula: tumour volume (mm^3) = $L \times W^2/2$ (where L is the length and W is the width) with the final measurement taken 4 weeks after tumour cell inoculation. At the same time, the body weight of each animal was measured once every 3 days, although they were more frequently checked during the first 3 weeks to monitor potential drug-related toxicity. At the end of the experiment (4 weeks after cell inoculation), the animals were anaesthetized by CO_2 and killed. Tumours from each animal were removed, measured and weighed individually (Kuo *et al.*, 2006; Yang *et al.*, 2008).

Statistical analysis. Each value represents mean ± SD. The control and experimental animal groups were compared by Student's *t*-test, $^{***}p < 0.001$ was considered significant.

RESULTS AND DISCUSSION

The results indicated that curcumin and doxorubicin decreased tumour size significantly. An illustration of a representative animal treated with curcumin relative to the control is shown in Fig. 2A. Curcumin treatment decreased significantly both tumour volume (Fig. 2B) and tumour weight (Fig. 2C) compared with the control. The percentage inhibition of each is shown in Table 1. None of the treatments, i.e. vehicle (DMSO), 30 mg/kg curcumin, 45 mg/kg curcumin or 8 mg/kg doxorubicin, altered the body weight significantly (data not shown). All tumours appeared only at the inoculation sites.

Based on these *in vivo* experiments, it can be seen that curcumin at 30 mg/kg can inhibit tumour growth in a NCI-H460 xenograft mice model. However, other investigators have shown that in human clinical trials, curcumin can safely be administered at doses up to 10 g/day. When given at 8 g/day, the serum concentration of curcumin was 1.77 ± 1.87 μmol/L, and there was no indication of dose-limiting toxicity (Cheng *et al.*, 2001). In the present study, serum concentrations of curcumin

Table 1. Inhibitory effect of curcumin on growth of H460 tumour xenografts in BALB/$c^{nu/nu}$ mice

Treatment	Tumour weight (g)	Inhibition (%)
Control	0.097	–
Curcumin 30 mg/kg	0.076	21.60
Curcumin 45 mg/kg	0.066[a]	31.96
Doxorubicin 8 mg/kg	0.028[a]	71.13

Doxorubicin and curcumin groups were compared and analysed using Student's *t*-test. [a] $p < 0.001$.

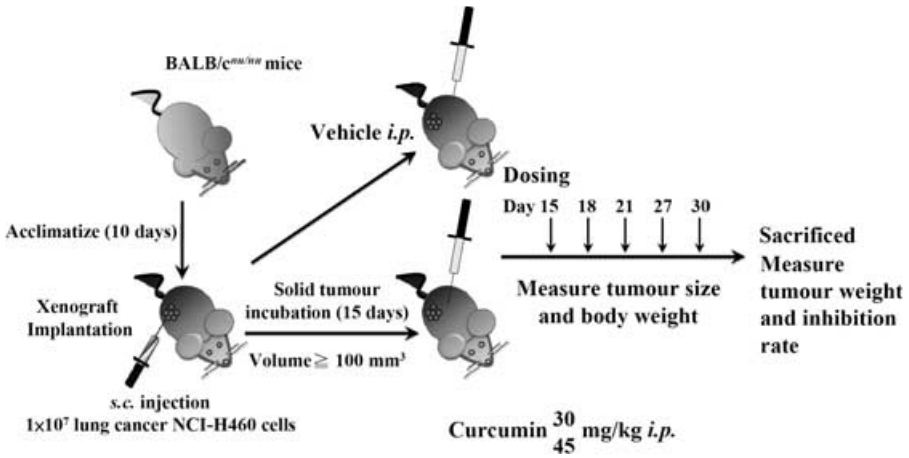

Figure 1. Experimental design of the xenograft animal model. Animals are implanted s.c. with NCI-H460 cells and when the tumour volume is around 100 mm^3, randomly divided into four groups and treated as described in Materials and Methods.

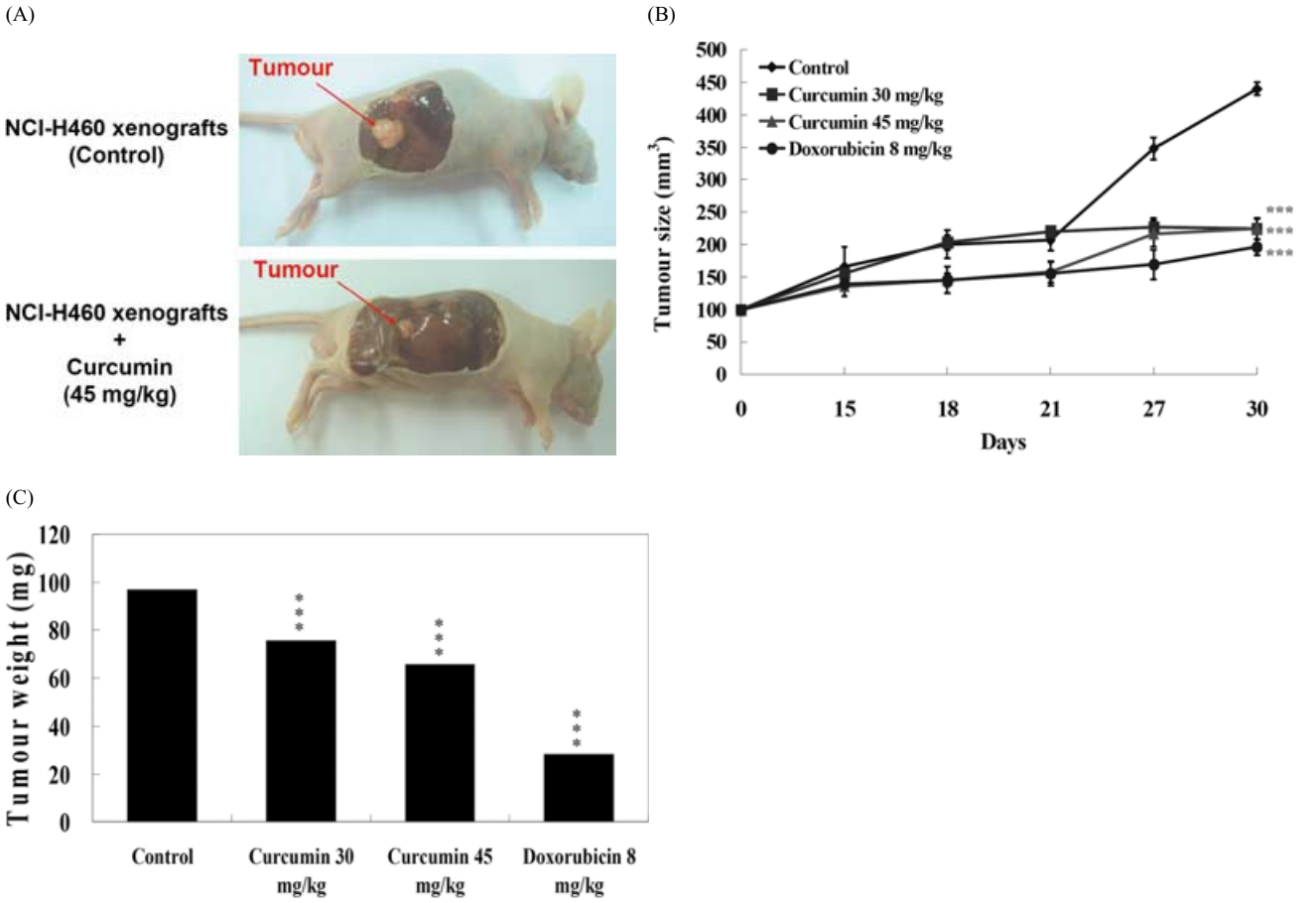

Figure 2. Effect of treatment on tumour growth. (A) Illustration of a representative tumour after treatment with curcumin and control. (B) The effect of curcumin and doxorubicin on tumour size. (C) The effect of curcumin and doxorubicin tumour weight. Data presented are the mean ± SD at 10–34 days post-tumour implantation; groups were compared and analysed using Student's *t*-test. *** $p < 0.001$.

and its metabolites were not measured, but despite the low bioavailability of curcumin, tumours in mice that received curcumin alone were about 55% smaller than those of the control group (Fig. 2B).

Even in the curcumin treatment (30 and 45 mg/kg) and doxorubicin (8 mg/kg) groups, tumours continued to grow slowly compared with the control group, indicating that complete regression of NCI-H460 cells xenografts was not achieved using a single treatment agent, suggesting that multiple treatments may be necessary to achieve a complete response. However, several recent reports have shown that combinations of curcumin with other agents can produce enhanced effects. Curcumin and genistein show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides (Verma *et al.*, 1997), and curcumin and phenethyl isothiocyanates, either alone or in combination, possess significant cancer-preventive activities in the PC-3 prostate tumour xenografts (Khor *et al.*, 2006). Curcumin potentiates the antitumour effects of gemcitabine in pancreatic cancer by suppressing proliferation, angiogenesis, NF-κB and NF-κB–regulated gene products (Kunnumakkara *et al.*, 2007). A combination of curcumin and light therapy increases the efficacy of curcumin in a human epithelial carcinoma A431 xenograft tumour model (Dujic *et al.*, 2009) and offers a new therapeutic concept. The present study provides the first report of the efficacy of curcumin against tumours in an *in vivo* xenograft of human lung cancer NCI-H460 cells in mice.

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