SHORT COMMUNICATION

Indonesian Tea Mistletoe (Scurrula oortiana) Stem Extract Increases Tumour Cell Sensitivity to Tumour Necrosis Factor Alpha (TNFα)

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The water extract of Indonesian tea mistletoe, Scurrula oortiana (‘benalu teh’), has been used for generations to treat tumours, however, little is known of its biological action. In the present study the stem and leaf extracts of S. oortiana were investigated for their modulation of tumour cell sensitivity toward TNFα, a potent anti-tumour cytokine. WEHI-164 cells which are known to be sensitive to TNFα, were used as a model. The assay results showed that either the stem or leaf extract of S. oortiana increased the sensitivity or susceptibility of WEHI-164 cells to TNFα as shown by decreases in LD₅₀ values in the TNFα sensitivity assay. The stem extract showed a greater increase than the leaf extract, increasing the sensitivity more than 160 times compared with the normal untreated tumour cells. This study showed for the first time that water extracts of S. oortiana were significantly cytotoxic to the WEHI-164 tumour cell line and increased tumour cell sensitivity to TNFα mediated lysis. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: Scurrula oortiana; Indonesian tea mistletoe; benalu teh; WEHI-164; TNFα.

INTRODUCTION

Indonesian tea mistletoe (‘benalu teh’) (Family Loranthaceae) is a parasitic plant that grows on tea trees. In Indonesia, especially in Java, it potencies as an (indigenous) anti-tumour agent has been known for generations and it has been used as a water extract by the local people. In spite of the knowledge and its use, there is very little scientific research substantiating the potency of the extract as a natural anti-tumour agent. Several studies on different species of the plant showed that methanol and water extracts of Scurrula parasitica L. did not inhibit the growth of cultured murine and human tumours, while methanol extracts of Scurrula fusca Bl. could inhibit the incorporation of 1H-leucine in Ehrlich ascites tumour cells (Sugeng, 1998; Shibuya et al., 1999). Another study of a whole plant extract of Loranthus parasiticus showed an inhibitory activity on avian myeloblastosis virus-reverse transcriptase (Kusumoto et al., 1992). From these few studies, the anti-tumour potency of the plant extracts remains elusive as does its mechanism. However, the testimony of the tumour sufferer who experienced complete regression after taking the extract continuously lends a plausible mechanism by which the body’s immune system is capable of fighting tumour cells.

One of the proinflammatory cytokines, i.e. tumour necrosis factor alpha (TNFα), is known to cause necrosis to tumour cells and is cytotoxic to a number of transformed cells. TNFα is one of the effector molecules used by macrophage and T-cells to fight tumour cells. Its clinical use in cancer treatment is limited due to cachexia at the dose required for tumour killing in vivo. The use of TNFα is confined to isolated limb perfusions (deVries et al., 1998; Eric et al., 1998; Plaat et al., 1999).

The present study was designed to investigate if water extracts of Indonesian tea mistletoe, Scurrula oortiana (Korth) Daus, Loranthaceae, can modulate the response of tumour cells toward TNFα.

MATERIALS AND METHODS

Plant material. S. oortiana was collected from the Center of Tea and Quinine Research, Ciwedey, West Java. The collected materials were taken to Herbarium Bogorensis for species identification (collection no. 26250, collector: Bacher).

Extraction. Five grams of dried leaves or stem were each extracted with 100 mL of water three times under reflux for 3 h. The resulting filtrates were evaporated to give dried extracts.

Preparation of samples. Each sample was dissolved in phosphate buffered saline (PBS) to give a 50 mg/mL concentration. Each sample was then filter sterilized for use in tissue culture.
Cell culture. WEHI-164 cells are a murine fibrosarcoma cell line which is sensitive to TNFα and used as a model of tumour cells. The cells were cultured in RPMI 1640 (Sigma) medium supplemented with 10% fetal bovine serum (Gibco), 10 µg/mL streptomycin (Sigma) and 100 U/mL penicillin G and incubated under standard cell culture conditions (Murwani and Armati, 1998). The cells used were more than 99% viable.

MA-1 cells are a normal fibroblast cell line which was derived from primary fibroblasts after a few passages (Murwani and Armati, 1998). They are used as a control of normal cells. The cells were cultured in EMEM (Sigma) medium supplemented with 10% fetal bovine serum (Gibco), 10 µg/mL streptomycin (Sigma) and 100 U/mL penicillin G and incubated under standard cell culture conditions.

TNFα sensitivity assay. The method was based on published TNFα toxicity assay. The assay is used to measure the concentration of TNFα-containing medium (Lieberman et al., 1989; Murwani and Armati, 1998). In this assay WEHI-164 cells which are sensitive to TNFα were used as the target of TNFα mediated lysis on tumour cells. TNFα is cytotoxic causing the cells to undergo lysis. Based on the sensitivity of these cells to TNFα, addition of serial dilution of known TNFα standard will generate a standard curve which shows the relationship between TNFα standard concentration and percentage of cell lysis. From this curve the LD₅₀ value can be determined. When an unknown TNFα concentration of a solution is added to the cells, a certain percentage of the cells will die. This unknown concentration can be determined from the standard curve. This principle was used to investigate the effect of Indonesian benalu teh extract on the tumour cells WEHI-164. However, instead of measuring TNFα concentration in the supernatant of S. oortiana extract-stimulated cells, the change in the WEHI-164 cells response to TNFα after extract stimulation was determined. If the treatment changed the sensitivity or response of WEHI-164 cells to TNFα, it would be reflected in the change of LD₅₀ value.

Stimulation of WEHI-164 and MA-1 cells with S. oortiana extract. The cells were cultured in several 25 cm² T-flasks (Nunc) in the complete medium as above at 1 × 10⁵ cells/mL for 24 h. The extract was then added to the cells at 1 mg/mL based on a preliminary study. For control WEHI-164 cells without extract addition, PBS was added in place of the extract. After 24 h stimulation the viable cells were counted, rinsed and resuspended in fresh medium for use in the TNFα sensitivity assay. For each assay, two-fold serial dilutions of standard TNFα (Sigma) starting from 50 ng/mL were prepared in duplicate in 96 well flat-bottom plates (Falcon). Control wells which contained no TNFα served as a 100% viability control. WEHI-164 cells (50 µL) that had been rinsed were added to each well to give a final cell density of 1 × 10⁵ cells/mL. After 24 h incubation, 20 µL tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue or MTT, Sigma) was added to all the wells and incubated further for 4 h. 100 µL of 10% SDS was then added to each well to dissolve the blue crystal formed. Following an overnight incubation, the optical density of the wells was determined by a microplate reader (Multiskan MCC/340 P) at a test wavelength of 540 nm. The absorbance was expressed as the percentage of cell lysis. The LD₅₀ was calculated from the graph of the percent cell lysis versus TNFα concentration.

RESULTS AND DISCUSSION

The results shown in Fig. 1 showed that extracts of S. oortiana leaves or stems had a direct cytotoxic effect on WEHI-164 cells. Microscopic observation and cell viability count using trypan blue indicated that at a cell density of 1 × 10⁵ cell/mL, the extract caused direct lysis up to 50% of the cell population. At lower cell densities, most cells were lysed (uncountable by trypan blue method). It is shown in Fig. 2 that after S. oortiana extract treatment the remaining viable cells became more sensitive or susceptible to TNFα mediated lysis compared with control without the plant extract. The stem extract showed a greater potency than the leaf extract in changing the sensitivity of WEHI-164 cells to TNFα. Treatment with the leaf extract changed the sensitivity of WEHI-164 cells to TNFα mediated lysis about 2-fold, while treatment with the stem extract changed the sensitivity more than 160 fold compared with the control cells without extract treatment. This result is interesting as treatment of normal fibroblast (MA-1 cells) with plant extracts showed no observable adverse effects. Normal fibroblasts retained their characteristic morphology as adherent and flattened cells with irregular processes that interconnected with neighbouring cells (Murwani and Armati, 1998). There was no giant cell formation and

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The viability of stimulated or control normal fibroblasts were comparable at near 100%. This may indicate that the extract has no side effects in normal cells.

This result has important implications as an increase in the sensitivity of tumour cells to TNFα may increase the ability of TNFα mediated killing by the immune cells and may explain the success of tumour elimination in a tumour sufferer taking the water extract of *S. oortiana* (combined leaves and stem together). It may also circumvent TNFα cytotoxicity for *in vivo* treatment by reducing the dose of TNFα. Further study of modulation of various tumour types toward TNFα after extract treatment will assist in elucidating this possibility. Isolation of the active constituents of the extracts responsible for increase sensitivity to TNFα mediated lysis is in progress.

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**REFERENCES**

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