

POTENTIAL ANTICANCER ACTIVITY OF TURMERIC (*CURCUMA LONGA*)

RAMADASAN KUTTAN, P. BHANUMATHY, K. NIRMALA and M.C. GEORGE

Amala Cancer Research Centre, Amala Nagar, Trichur-680 553, Kerala (India)

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SUMMARY

Anticancer activity of the rhizomes of turmeric was evaluated in vitro using tissue culture methods and in vivo in mice using Dalton's lymphoma cells grown as ascites form. Turmeric extract inhibited the cell growth in Chinese Hamster Ovary (CHO) cells at a concentration of 0.4 mg/ml and was cytotoxic to lymphocytes and Dalton's lymphoma cells at the same concentration. Cytotoxic effect was found within 30 min at room temperature (30°C). The active constituent was found to be 'curcumin' which showed cytotoxicity to lymphocytes and Dalton's lymphoma cells at a concentration of 4 µg/ml. Initial experiments indicated that turmeric extract and curcumin reduced the development of animal tumours.

INTRODUCTION

Turmeric (*Curcuma longa*) is a common spice used daily in curries in South India. Moreover, turmeric has been prescribed for many ailments in Indian natural medicines [4]. It is applied to fresh wounds and bruises and also acts as a counter-irritant in insect bites. Internally it is an anthelmintic. Turmeric paste is applied to facilitate the process of scabbing in chicken pox and small pox. It is also used in many urinary diseases, diseases of liver and in jaundice. Turmeric has been described as a cancer remedy in Indian natural medical literature. However, no systematic effect has been made to study its effects by modern techniques. We present here that turmeric extract and curcumin isolated from it is highly cytotoxic to mammalian cells and is effective in reducing animal tumours, indicating its potential for use in cancer treatment.

MATERIALS AND METHODS

Turmeric rhizomes were powdered and passed through a no. 400 sieve. One gram of powder was extracted with 200 ml of 50% ethanol and kept overnight. The extract was filtered and dried. The dried extract was reconstituted in 5 ml of 50% ethanol. A small volume (500 μ l) was diluted to 5 ml in normal saline. Aliquots of the same (500 μ l to 1 μ l) in 1 ml saline were used to test the cytotoxicity. All the values reported here are expressed in terms of original turmeric powder. Curcumin isolated from turmeric was a gift from Dr. S.B. Rao. Twenty milligrams of curcumin was dissolved in 5 ml ethanol. An aliquot (500 μ l) was diluted with 4.5 ml normal saline. Aliquots of the same were used to study the cytotoxicity. CHO cells (from the National Institute of Virology, Pune, India) were grown in Eagle's minimum essential medium with glutamine and bicarbonate in the presence of 10% fetal calf serum. Lymphocytes were prepared using the Ficoll-Hypaque method [3] from either normal donors or from patients with leukemia. Dalton's lymphoma cells grown in the peritoneal cavity of mice were aspirated and washed in normal saline. These cells were used either for cytotoxicity experiments or for injecting into fresh mice.

Cytotoxicity experiments

Lymphocytes from normal donors or patients with leukemia and ascites cells from mice were used for the cytotoxicity experiments. The cells were diluted to approximately 1 million cells/ml in normal saline. Turmeric extract was added to this cell suspension at various concentrations such as 10 mg to 0.02 mg per ml. When curcumin was used, the concentrations ranged from 200 μ g to 0.5 μ g per ml. The cells were normally incubated for 3 h at room temperature (30°C). After the incubation, trypan blue (0.4%) was added and the percentage of dead cells was determined using a haemocytometer.

The growth inhibition of CHO cells was determined by adding aliquots of turmeric extract (10–0.02 mg/ml) to the cells at logarithmic phase. After 5 days the cells were trypsinized and counted.

Animal experiments

Mice were given Dalton's lymphoma cells (1 million) intraperitoneally. Turmeric extract at various concentrations (10–40 mg/animal) were given to a group of mice ($N = 8$) through the same route for 0–10 days. The controls received saline. Due to the insolubility of curcumin in aqueous materials it was not possible to make a uniform solution of curcumin. Although neutralization of phenolic groups of curcumin with sodium hydroxide made curcumin soluble, it reduced the cytotoxicity. In order to uniformly suspend curcumin we employed liposomal encapsulation. Neutral liposomes of curcumin (5 mg/ml) were prepared by standard procedures involving phosphatidyl choline and cholesterol. Unilamellar liposomes were prepared by sonication. An aliquot of the liposome containing

1 mg, i.e. (50 mg/kg) was given i.p. to mice the day after giving the Dalton's lymphoma cells and continued for 10 days. After 30 days and 60 days the number of surviving animals was counted. Tumour development was also noted both in controls and in experimental animals.

Paper chromatography of turmeric extract and that of curcumin was done using Whatman No. 1 filter paper with benzene as the solvent system.

RESULTS

Turmeric extract was found to be highly cytotoxic to normal human lymphocytes, to human leukemic cells and to Dalton's lymphoma cells (Table 1). The turmeric exhibited its cytotoxicity at concentrations of 0.4 mg/ml and above. Since the active component is only a small fraction of the original, the cytotoxic level is highly remarkable. There was no difference in the concentration of turmeric extract effective against normal or leukemic lymphocytes as well as Dalton's lymphoma cells.

Turmeric extract also inhibited the growth of CHO cells at concentrations of 0.4 mg/ml and above. In order to determine the time taken for cytotoxicity, Dalton's lymphoma cells were treated over various time intervals (15 min to 3 h) with 3 concentrations of turmeric extract (4, 2, 1 mg/ml), and the percentage of dead cells was assessed by trypan blue uptake. It was found that in all these concentrations turmeric showed its activity as early as 30 min at room temperature.

Initial experiments using various solvent systems to extract the cytotoxic principle indicated that benzene, chloroform, acetone, ethyl acetate and ethanol could extract the cytotoxic principle, while water could extract only a minimum activity. Using a consecutive extraction with various solvent systems it was found that extraction of benzene followed by ethyl acetate extracted most of the cytotoxic principles and further extraction with chloroform, acetone, ethanol and 50% ethanol did not extract any more cytotoxic materials. Paper chromatography of benzene extracts of turmeric and the cytotoxic activity of various cut strips indicated that cytotoxicity was clearly associated with the yellow colouring material of turmeric extract.

TABLE 1

CYTOTOXICITY OF TURMERIC EXTRACT AND CURCUMIN

Drug tested	Minimum amount of the drug/ml producing 100% cell death ^a		
	Normal Lymphocytes	Leukemic Lymphocytes	Dalton's lymphoma cells
Turmeric extract	1 mg	1 mg	1 mg
Curcumin	8 µg	8 µg	4 µg

^aIncubation was carried out at 30°C for 3 h.

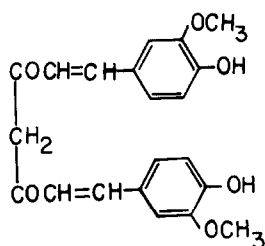


Fig. 1. Structure of curcumin.

This yellow colouring material was found to be curcumin (Fig. 1) which moved with the cytotoxic component.

We have also tested the curcumin for its cytotoxicity and found that curcumin was cytotoxic to lymphocytes and Dalton's lymphoma cells at concentrations of 1–4 $\mu\text{g/ml}$ and above (Table 1).

We have done some preliminary experiments on the use of turmeric extract and curcumin in preventing animal tumours induced by Dalton's lymphoma. As shown in Table 2 there was a significant reduction in the death and tumour formation in animals receiving 40 mg of turmeric. Fifty percent of the animals in this group survived more than 60 days and these animals were tumour free. The anticancer effect was reduced when the dose of the drug was decreased. There was no toxicity at the 40 mg dose.

TABLE 2

EFFECT OF TURMERIC EXTRACT AND LIPOSOMAL ENCAPSULATED CURCUMIN ON TUMOUR FORMATION AND SURVIVAL OF ANIMALS

Dosage	Tumour formation 30 days (%)	Animals survived after 30 days (%)	Animals survived after 60 days (%)
<i>Turmeric extract</i>			
Control			
Non-treated ($N = 8$)	100	25 (2/8)	0 (0/8)
From 40 mg of turmeric ($N = 8$)	12	75 (6/8)	50 (4/8)
From 20 mg of turmeric ($N = 8$)	50	50 (4/8)	12 (1/8)
From 10 mg turmeric ($N = 8$)	75	25 (2/8)	12 (1/8)
<i>Liposomal encapsulated curcumin</i>			
Curcumin (1 mg) ($N = 5$)	40	100 (5/5)	60 (3/5)
Control (non-treated) ($N = 5$)	100	0 (0/5)	0 (0/5)

Due to the small amount of ethanol present in the extract and the insolubility of active ingredients in aqueous medium concentration more than 40 mg/animal could not be administered. When curcumin was used as liposomes at concentrations of 1 mg/animal, all the animals survived 30 days and only 2 of the animals developed tumours and died before 60 days.

DISCUSSION

The results indicate that turmeric extract is a potential anticancer agent. The anticancer property is possibly associated with the curcumin, the cytotoxic component present in turmeric. The *in vitro* experiments indicate that curcumin is cytotoxic at concentrations of 1–4 $\mu\text{g/ml}$ whereas turmeric is cytotoxic at concentrations of 400 $\mu\text{g/ml}$. This was as expected as the curcumin concentration in the turmeric is only 1% [2]. The animal experiments indicate that turmeric extract and curcumin decreased the incidence of tumour formation in experimental animals. However, this aspect of the study needed more work using different types of tumour. It has been previously demonstrated that curcumin is bactericidal to *Micrococcus pyogenes* at concentrations of 1:1000000 [6]. In our experiments using mammalian cells the cytotoxic level is very close to its bactericidal action.

The essential oil isolated from turmeric was also found to have bactericidal action but was found to be less than that of curcumin [8]. A known anthelmintic, santonine is also present in turmeric [9]. Other than these actions, curcumin and sodium curcumate were found to act as a cholorectic and hydrochologogic agent [7]. It increases bile secretion and decreases its solid content. Moreover, it has also been reported to reduce cholesterol in dogs when given orally [5].

At present we do not know the mechanism of action of turmeric or curcumin in the cells. Addition of turmeric has been shown to arrest mitosis, altered chromosome morphology and interfered with nucleic acid synthesis. The metaphase chromosomes showed a progression of changes including uncoiling, chromatid separation, fragmentation and disintegration. This progression was dose- and time-dependent [1]. The immediate mitotic arrest induced by turmeric suggest that this spice affects centrioles and spindle proteins as in the case of other mitotic poisons. Light microscopic studies indicate that the yellow component enters the cells before the rupture of the cell membrane. It is also possible that the dye may be acting directly at the cell membrane. Curcumin due to its conjugated diene structure may also participate in the biological oxidation mechanisms.

Curcumin was found to be a non-toxic compound when given orally to humans as a drug for arthritis (S.B. Rao, pers. commun.). This aspect of the study using an animal model will be published elsewhere. More work is needed to reveal the potential activity of this interesting compound isolated from this herb which is very commonly used in India and other Asian countries as a medicine and culinary agent.

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