Flavonoids Protect Against T-2 Mycotoxins both *in vitro* and *in vivo*

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Quercetin was able to reduce the cytotoxic effect of T-2 mycotoxin on cultured murine thymocytes. When given to mice immediately before challenge with T-2 mycotoxins, quercetin significantly reduced mortality.

INTRODUCTION

Trichothecene mycotoxins are a chemically related group of secondary metabolites of *Fusarium* and some other fungi which are toxic in both man and animals. Mycotoxins have been implicated as the cause of inadvertent food intoxication following fungal contamination of foodstuffs (Greenway & Puls, 1976; Hsu *et al.*, 1972; Ueno *et al.*, 1972) and recent studies have suggested their use as biological warfare agents (Mirocha *et al.*, 1983; Rosen & Rosen, 1982).

While some compounds are beneficial in treating mycotoxin poisoning, there is no widely accepted treatment. Anecdotal reports from South East Asia suggest that extracts of plants rich in bioflavonoids can be successful in the treatment of mycotoxicosis. Bioflavonoids are common constituents of many plants and are widely distributed in nature. They have a variety of effects on mammalian tissue and organ systems (Willaman, 1955). Quercetin (3,3',4',5,7-penta-hydroxyflavone), one such bioflavonoid compound, has received considerable attention for its inflammatory effects and influence on other organ systems (Middleton *et al.*, 1985).

Previous work from this laboratory has shown that mycotoxins, in particular T-2 mycotoxin, have potent cytotoxic capabilities *in vitro*, expressed as a loss of viability of thymocytes and other leucocytes (DiNinno *et al.*, 1985). Here we describe the effect of quercetin on this *in vitro* cytotoxicity and on the lethal effects of T-2 mycotoxin on mice.

METHODS

Cell cultures. Isolated thymus cells were prepared as described by Shiigi *et al.* (1980). Black male C-57 mice 3–6 weeks old, were used in all but one experiment where 24-week-old mice were used. The mice were killed by CO₂ inhalation and the thorax was flooded with 70% (v/v) ethanol prior to removal of the thymus. For each assay, at least two thymi were collected. After removal, the thymi were placed in a Petri dish containing Joklik's Medium (Flow Laboratories) and minced with scissors and teased apart to dissociate cells. The cell suspension was washed by centrifugation (400 *g* for 20 min at 25 °C) and the pellet was resuspended in the same medium to give a concentration of 2×10^7 cells ml⁻¹.

Mycotoxins. T-2 mycotoxin (Sigma) was dissolved in dimethylsulphoxide (DMSO) to give a stock solution of 20 mg ml⁻¹. Further dilution in DMSO allowed addition of specified amounts of mycotoxin to 0.5 ml cell suspension (1×10^7 cells). The amount of DMSO added did not exceed 25 µl and controls, containing cells and DMSO alone, were tested to detect any inherent cytotoxicity of DMSO.

Cytotoxicity assay. Cytotoxicity was assessed by determining the viability of thymocytes using dye exclusion with 0.2% eosin (Shiigi *et al.*, 1980). At specified times after addition of T-2 mycotoxin to the cells, a portion of the

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Abbreviation: DMSO, dimethylsulphoxide.

Fig. 1. Protective effect of quercetin on cytotoxicity of T-2 mycotoxin on murine thymocytes. ●, Control cells incubated with DMSO. ■, Cells incubated with 400 μg T-2 mycotoxin ml^{-1} (a), or 200 μg T-2 mycotoxin ml^{-1} (b). ▲, Cells incubated with 400 μg T-2 mycotoxin ml^{-1} plus 10 μg quercetin ml^{-1} (a) or 200 μg T-2 mycotoxin ml^{-1} plus 10 μg quercetin ml^{-1} (b). Data represent means $\pm$ SD of at least four separate experiments. $P \leq 0.02$ at 2 h (a) or 3 h (b) or more as determined by Student's *t*-test.

mixture was removed and mixed with an equal volume of eosin solution. Cells were examined microscopically in a Neubauer haemocytometer and the viability of at least 200 cells was determined.

Quercetin (Sigma) was dissolved in DMSO and added to the T-2 mycotoxin/thymocyte mixture at specified times. Controls were included to determine if quercetin and additional DMSO had a cytotoxic effect on the thymocytes. In other experiments, quercetin was added before or after introduction of T-2 mycotoxin into the thymocyte culture.

In vivo protection. Mice were challenged by intraperitoneal injection of 4 mg T-2 mycotoxin (dissolved in DMSO) kg^{-1} . This dose represented approximately 1 MLD. Mice receiving quercetin were given the drug immediately before (within 15 min) injection of T-2 mycotoxin. Quercetin was prepared for injection by entrapment in phospholipid vesicles (liposomes). Quercetin was dissolved in absolute ethanol and DMSO (10:1, v/v) to give a solution of 40 mg ml^{-1} and 450 μl was placed in a test tube. An equal volume of egg-yolk phosphatidylcholine (Sigma) (100 mg ml^{-1} in chloroform/ethanol) was added and the mixture was dried under N_2 and subsequently in a vacuum desiccator. Saline (2 ml) was added to the dried film and the test tube was shaken vigorously to form multilamellar vesicles (MLV). The liposome suspension was diluted with saline to a total volume of 20 ml. This was given to mice at concentrations of 10–20 or 20–30 mg quercetin per ml of liposome suspension.

RESULTS AND DISCUSSION

In Fig. 1 it is apparent that quercetin offered significant protection against cytotoxicity. At the concentrations of T-2 mycotoxin tested, quercetin reduced the cytotoxic effects on thymocytes by approximately 40%. This protection was greatest when the drug was added before or at the same time as addition of T-2 mycotoxin to the thymocyte cultures (Fig. 2). Delaying the addition of quercetin by 2 h significantly reduced its protective effect. When quercetin was given to mice just before lethal challenge with T-2 mycotoxin, significant protection was obtained at both 24 and 48 h after challenge (Table 1). Apparent differences between quercetin treatment doses were not significant upon chi-square analysis. Quercetin at lower doses, or liposomes themselves, had no protective effect (data not shown).

It has been suggested that inhibition of protein synthesis and, to a lesser degree, inhibition of DNA and RNA synthesis is responsible for the observed toxic effects of T-2 mycotoxin (Oldham *et al.*, 1980; Ueno *et al.*, 1973). More recent studies, however, have focused on alterations in cell membranes as mechanisms of cytotoxicity and pathological consequences

Fig. 2. Effect of the time of addition of quercetin on the protection from cytotoxicity of T-2 mycotoxin on murine thymocytes. Cells were mixed with 200 μg T-2 mycotoxin ml^{-1} at 0 h and 10 μg quercetin ml^{-1} was added at the times indicated. Cytotoxicity assays were done 5 h after the addition of T-2 mycotoxin. Except for -2 h, which was a single trial, the data represent means $\pm$ SD of at least three separate experiments.

Table 1. *Effect of quercetin on T-2-mycotoxin-induced mortality in mice*

Mortality is expressed as (no. mice dead)/(no. mice challenged), and as a percentage in parenthesis. Both quercetin groups showed significant reduction in mortality ($P < 0.002$) when data was subjected to chi-square analysis.

Dose of quercetin (mg kg^{-1})	Mortality	
	24 h	48 h
0	48/72 (67)	64/72 (89)
10-20	13/53 (25)	25/53 (47)
20-30	11/36 (31)	21/36 (58)

seen in mycotoxin poisoning (Gyongyossy-Issa *et al.*, 1985; Rottem *et al.*, 1984; Scappert & Khachatourians, 1984; Segal *et al.*, 1983).

Quercetin is among a large number of flavonoid compounds commonly found in a variety of fruits and vegetables (Kühnau, 1976). These flavonoid compounds have manifold biological properties and have been used as diuretics, heart stimulants, antiviral agents, antitumour agents and antihistaminics (Willaman, 1955). Quercetin itself has anti-inflammatory properties, inhibits NADPH oxidase in neutrophils and will decrease spontaneous lipid peroxidation following glutathione depletion in rats (Beretz *et al.*, 1982; Tauber, 1984; Younes & Sigers, 1981). While there is no clear indication of how quercetin protects against T-2 toxicosis, evidence implicating participation of cell membranes in the effects of T-2 mycotoxin suggests that activities relating to inhibition of lipid peroxidation or stabilization of cellular enzyme activation may be involved. As has been observed for related flavonoid compounds, quercetin may also activate degrading enzymes in the liver (Newberne, 1975). However, because of the ability of quercetin to protect *in vitro*, the former mechanism must also be relevant.

Other attempts to reduce the effect of T-2 mycotoxin poisoning have included the administration of zeolite or bentonite to reduce intestinal absorption, and the administration of inducers of microsomal enzymes or agents which increase hepatic glutathione levels (Carson & Smith, 1983; Fricke, 1983; Fricke *et al.*, 1984). It may be that quercetin or other flavonoid compounds share some of these activities and may be viewed as possible therapeutic agents.

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