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Short Communication

Quercetin potentiates TNF-induced antiviral activity

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Summary

Tumor necrosis factor (TNF) produces a dose-dependent inhibition of vesicular stomatitis virus (VSV), encephalomyocarditis virus (EMCV), and herpes simplex virus type 1 (HSV-1) replication in WISH cells. The antiviral activity of TNF against VSV and EMCV is greatly enhanced by combination with quercetin. Induction of 2′,5′-oligo-adenylate (2-5A) synthetase by TNF is also enhanced by quercetin. Addition of polyclonal antibodies to human interferon (IFN) -β completely blocked both enhancement of antiviral activity and 2-5A synthetase induction.

TNF; Quercetin; Antiviral effect; 2-5A synthetase

Tumor necrosis factor (TNF) is a cytokine produced mainly by activated macrophages (Carswell et al., 1975). It has been reported that TNF exerts a number of biological effects, and recently antiviral activity in several cell lines was also demonstrated (Wong et al., 1986; Mestan et al., 1986; Ruggiero et al., 1989a). The antiviral activity of TNF is strongly synergistic with interferons (IFNs) (Mestan et al., 1988; Wong and Goeddel, 1986; Feduchi et al., 1989). We have found that quercetin greatly enhanced the antiviral activity of recombinant human TNF-α. TNF-α used in these experiments was provided by Asahi Chemical Industry Co. (specific activity was 2.3×10^6 Japanese reference units/mg) in WISH cells (derived from human amnion tissue).

Quercetin is a bioflavonoid which is distributed widely in many fruits, vegetables and tea (Kuhnau, 1976). The biological effects of quercetin and

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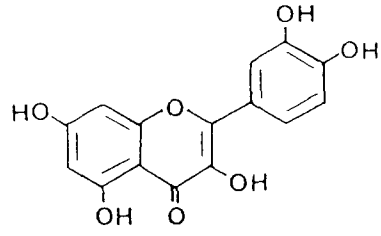

Fig. 1. Chemical structure of quercetin

some of its derivatives have been reported. For example: inhibitory effects on growth of malignant cells and glycolysis (Suolinna et al., 1975); macromolecule synthesis (Graziani et al., 1979); activity of protein kinase (Graziani et al., 1981); activity of ATPases (Kuriki et al., 1976); replication of viruses (Ishitsuka et al., 1982; Castrillo et al., 1986; Vrijsen et al., 1987); and induction of heat shock proteins (Hosokawa et al., 1990).

In this report we describe the effect of quercetin on TNF-induced antiviral activity. Quercetin was purchased from Extrasythese (Genay, France), and was dissolved in dimethyl sulfoxide (DMSO). The chemical structure of quercetin is shown in Fig. 1. Treatment of WISH cells with TNF produces a dose-dependent inhibition of virus replication (Fig. 2). TNF-induced antiviral activity against VSV and EMCV was greatly enhanced by combination with quercetin, whereas quercetin was not effective against TNF-induced anti-HSV activity. Maximal enhancement of TNF-induced antiviral activity was obtained with lower doses of TNF in combination with 10 μM of quercetin.

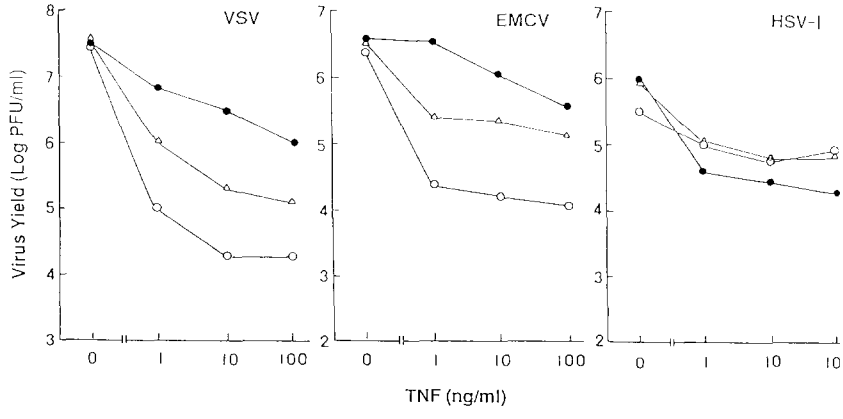

Fig. 2. Effect of quercetin on the antiviral activity of TNF. WISH cells were grown in multiwell plates (24 well) with Dulbecco's Eagle's medium containing 10% fetal bovine serum. Confluent cells were incubated for 20 h at 37° C with different doses of TNF alone (●) or in the presence of 5 μM quercetin (△) or 10 μM quercetin (○). Monolayers were washed and infected with VSV, EMCV or HSV-1 at MOIs of 3.0, 3.0, and 1.0, respectively. After 24 h (VSV and EMCV) or 48 h (HSV-1), virus yields were determined by plaque assay.

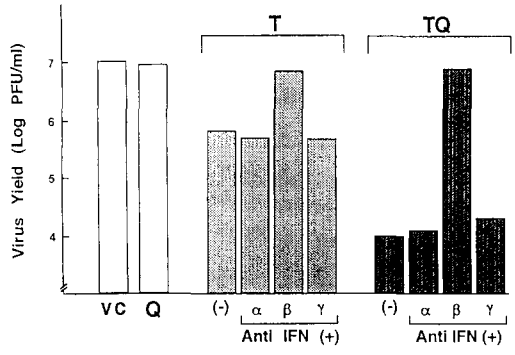

Fig. 3. Effect of antibodies to IFNs on TNF- or TNF/quercetin-induced antiviral activity. WISH cells were incubated for 20 h at 37°C with final concentration of 5 ng/ml TNF(T) or with 5 ng/ml TNF/10 μM quercetin (TQ) in the presence of polyclonal antibodies to human IFN-α, -β and -γ. These antibodies were used at concentrations that neutralized 5000 units of the corresponding IFN. Cells were then infected with VSV and incubated as described in Fig. 2.

Pretreatment by quercetin alone has not shown antiviral activity. The combined treatment did not increase cytotoxic activity, and an antiviral activity was not detectable in supernatants from TNF or TNF/quercetin-treated cultures (data not shown). The enhanced effect of quercetin on TNF-induced antiviral activity was not observed when TNF was added to the culture medium after washing cell monolayers that had been treated for 20 h with quercetin.

We investigated whether or not enhancement of TNF-induced antiviral activity is caused by other flavonoids; luteolin, kaempherol, genistein, and rutin. However, no enhanced inhibitory effect on VSV by combination of TNF with flavonoids was observed (data not shown).

Ruggiero et al. (1989a) have reported previously that the TNF-induced antiviral activity was abrogated by antibodies to IFN-β on WISH cells. It was suggested that TNF action might involve induction of IFN-β. Therefore we investigated whether anti-human IFNs antibodies inhibit the antiviral activity induced by combined treatment of TNF and quercetin. Polyclonal antisera to IFN-α and IFN-γ were prepared by immunizing rabbits with human recombinant IFN-α 2a (Nippon Roche, specific activity was $>10^8$ IU/mg) and human recombinant IFN-γ (Shionogi, specific activity was 4.5×10^6 Japanease reference units/mg), respectively. Polyclonal rabbit antibodies to human IFN-β were obtained from Toray industries, INC. As shown in Fig. 3, antibodies to IFN-β completely inhibited the TNF- or TNF/quercetin-induced antiviral activity. Anti-human IFN-β antibodies have no cross-reactivity with recombinant human TNF-α. These results show that a TNF- or TNF/quercetin-induced antiviral state is mediated through the induction of IFN-β.

On the other hand, it has been shown that TNF treatment leads to an increase of 2-5A synthetase levels (Ruggiero et al., 1989a) in WISH cells. We also examined the 2-5A synthetase activity after treatment with TNF and TNF/

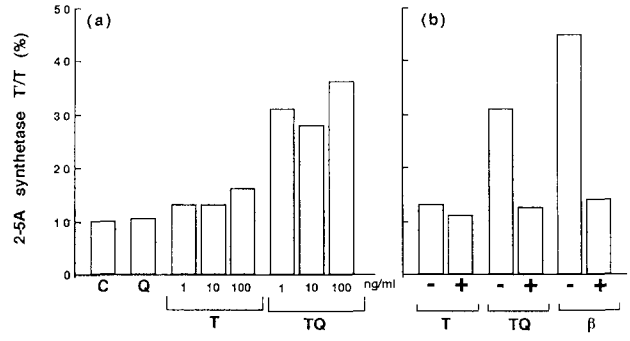

Fig. 4. Induction of 2-5A synthetase activity (a) and effect of antibodies to IFN-β (b). (a) WISH cells were incubated for 20 h at 37°C with different doses of TNF(T) or/and 10 μM quercetin (Q). Untreated cells were used as a control (C). The assay for measuring 2-5A synthetase activity was performed as described previously (Ohnishi et al., 1986). (b) WISH cells were incubated for 20 h at 37°C with 1 ng/ml TNF(T), 1 ng/ml TNF/10 μM quercetin (TQ) and 20 IU/ml IFN-β (β) as a positive control in the absence (−) or presence (+) of polyclonal antibodies to IFN-β. The activity of 2-5A synthetase was measured.

quercetin. As shown in Fig. 4(a), 2-5A synthetase activity was greatly enhanced in those cultures which were treated with both TNF and quercetin. However, as shown in Fig. 4(b), the activity was annihilated when antibodies to IFN-β were added. These results demonstrate that induction of 2-5A synthetase by TNF or TNF/quercetin was mediated through the TNF-induced IFN-β. We also found that quercetin enhanced the antiviral activity induced by interleukin (IL)-1 in WISH cells (data not shown). It has been reported that IFN-β is a mediator of the antiviral effect of IL-1 (Vam Damme et al., 1987; Ruggiero et al., 1989b). These findings show that TNF and IL-1 exert their antiviral effects through a common mediator, namely IFN-β.

Although the data presented here do not directly demonstrate TNF-induced IFN-β, it is suggested that quercetin potentiates the antiviral action of TNF by enhancing the action of IFN-β and/or by increasing the production of IFN-β.

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