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Synergistic action of quercetin and murine alpha/beta interferon in the treatment of Mengo virus infection in mice

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Summary

$ABD2F_1$ mice were infected intraperitoneally (i.p.) or intranasally (i.n.) with Mengo or Sindbis virus and treated with either crude murine alpha/beta interferon (MuIFN-α/β) or quercetin, or both. MuIFN-α/β given i.p. or intramuscularly (i.m.) 1–3 h before the infection had a dose-dependent protective effect regardless of the route of administration. When given after the infection, IFN did not show any effect. Oral quercetin, capable of protecting cardio, i.e. Mengo virus-infected mice, failed to show antiviral efficacy in Sindbis virus-infected animals. Of various combinations of quercetin and MuIFN-α/β, a certain well defined regimen resulted in a significant enhancement of protection in Mengo, but not Sindbis, virus-infected mice. A marginally effective treatment regimen of quercetin (20 mg/kg, given 12 h before Mengo virus infection, and 10 mg/kg given both 1 h before and 12 h after infection) potentiated the activity of a single dose of MuIFN-α/β (5000 IU 3 h prior to infection), giving 85–100% survivors compared to 50% for MuIFN-α/β when applied alone ($p < 0.001$).

Mengo virus; Sindbis virus; Mouse; Quercetin; MuIFN-α/β; Synergistic antiviral activity; Histology

Introduction

Picornavirus infections causing severe or widespread human diseases represent potential targets for antiviral chemotherapy since vaccination may be practical only for a few serological types, i.e. polioviruses 1–3 (De Clercq, 1985; Eggers, 1985;

Korant et al., 1984; Lambert, 1983; Prusoff et al., 1984). At present no antiviral agents are available for the treatment of human diseases caused by the entero- and rhinovirus members of the picornavirus family. Potent inhibitors of these viruses therefore deserve serious consideration.

Among the bioflavonoids and related compounds with proven antiviral activity in animal models (Cutting et al., 1953; Güttner et al., 1982; Ishitsuka et al., 1982; Veckenstedt and Psuztai, 1981; Veckenstedt et al., 1978) quercetin and 4,5-diacetyloxy-3,3′,7-trimethoxyflavone (Ro 09-0298) have been found to produce a significant protective effect in systemic picornavirus infections. Likewise, interferon has proven to be efficient against such diseases (Gresser et al., 1968; Olsen et al., 1976; Weck et al., 1982, 1983).

Combinations of antiviral compounds and interferon may produce enhanced efficacy against encephalomyocarditis virus infections in mice (Chany and Cerutti, 1977; Werner et al., 1976). These findings prompted us to investigate the antiviral potency of quercetin in combination with MuIFN-α/β in an experimental Mengo virus infection. For comparative reasons, Sindbis virus infection which is sensitive towards MuIFN-α/β, but not to quercetin, was investigated in parallel.

Materials and Methods

Mice

Specified pathogen free (SPF) male (AB/Jena × DBA/2 Jena)F_1-hybrids (ABD2F_1) were obtained from the mouse facilities of the Jena Institute. Conventionally bred male Lati:CFLP mice were obtained from the Animal Breeding Farm Lati, Gödöllö, Hungary. Animals, 4–6 weeks old, were housed in plastic cages under conventional conditions with standardized pellet food and tap water ad libitum.

Viruses

The $Mengo_M$ virus strain (Veckenstedt, 1974) and Sindbis virus, strain AR 86, neuroadapted to mice, were used. The virus stocks were prepared as previously described (Veckenstedt et al., 1985a). For i.p. or i.n. virus challenge the stocks were diluted in phosphate-buffered saline (PBS) to contain 10 LD_{50} per dose of injection. In Mengo virus-infected mice necrotic changes were present in the central nervous system, salivary and lacrimal glands, thymus and pancreas, and less severe changes were seen in the kidneys (Güttner et al., 1982; Schmidt et al., 1984; Zschiesche and Veckenstedt, 1974). Sindbis virus induced similar lesions and, additionally, moderate alterations in the liver cells (Veckenstedt et al., 1985b).

The Cantell strain of Sendai virus grown in SPF eggs served for induction of MuIFN-α/β. The Indiana strain of vesicular stomatitis virus (VSV) was used as the challenge virus of IFN titrations in L_{929} cells.

Antiviral agents

Cristalline quercetin (mol. weight 338.27) was obtained from Merck, Darm-

stadt, F.R.G. (batch No. 75 21550). Drug suspensions were prepared immediately before oral administration (Veckenstedt et al., 1978).

Serum containing Sendai virus-induced MuIFN-α/β was used for the experiments. Samples with titres of 4.7 – 4.9 $\log_{10}$ international units (IU) per ml were diluted to the desired concentrations with physiological saline. Mock MuIFN was prepared from the serum obtained from normal mice.

Interferon assay

MuIFN-α/β preparations were assayed in a microtitre assay with L_{929} cells using VSV as the challenge virus. In each assay an internal laboratory standard was included which had been calibrated against the international reference preparation of MuIFN (G 002-904-511, NIH, Bethesda, MD, U.S.A.).

Assay of antiviral effectiveness

Groups of ten or 20 mice were injected i.p. or i.n. with 10 LD_{50} of the virus, causing approximately 96% mortality. Quercetin was administered orally at various doses using treatment schemes specified below. As placebo, drug diluent was used instead of quercetin (Veckenstedt et al., 1978).

Single injections of serum containing various concentrations of MuIFN-α/β were injected i.p. or i.m. to mice prior to or after virus infection as indicated below. Mock MuIFN served as placebo.

The protective effects of different combinations of the two antiviral agents were evaluated in both Mengo and Sindbis virus-infected mice. Surviving animals were recorded twice daily for 14 days.

Histopathology

Mice that died during the experiments as well as survivors that were killed at the conclusion of the experimental period were thoroughly necropsied. The samples of the virus target organs were fixed by immersion in 4% buffered formalin and embedded in paraffin. Sections 5–6 μm thick were processed by standard methods and stained by haematoxylin and eosin.

Statistical evaluation and definition of drug synergism

The virus LD_{50} and the MuIFN-α/β ED_{50} were determined by the method of Reed and Muench (1938). Significance of survival was evaluated by the Fisher exact test at the 95% level. The interactive effect of drug combinations was determined by comparison of y_C with the product of y_A and y_B, where y_A are the fractional mortalities of drug A and drug B as single agents, and y_C is the fractional mortality of the drugs when used in combination. If $y_C < y_A + y_B$, the effect of the combination was regarded as synergistic and if $y_C > y_A + y_B$, the effect was considered antagonistic (Connell et al., 1985).

Results

Effect of quercetin

Quercetin was shown to cause a significant protective response in Mengo virus-infected $ABD2F_1$ mice (Veckenstedt et al., 1978). Experiments were performed to find marginally effective treatment regimens to be used in combination with MuIFN-α/β. At doses of 5–10 mg/kg the drug had no protective effect against the lethal disease when administered both 12 h and 1 h before and 12 h after infection.

Morphologically, Mengo virus-infected and quercetin-treated animals that died within a 2-week period after infection showed the same necrotic lesions as the virus-infected, placebo-treated controls. However, in the few infected, drug-treated survivors only slight changes in organs other than the brain were found. These results agree with earlier findings (Güttner et al., 1982).

$ABD2F_1$ mice infected i.n. with 10 LD_{50} Sindbis virus were treated orally with 5, 10, 15, 20, 25 or 30 mg/kg quercetin at −12, −1, 8, 24, 32, 48 and 56 h, relative to infection. Virus controls were treated in the same manner with placebo. Under these conditions quercetin did not exhibit any efficacy against lethal Sindbis virus infection in mice.

The histologic findings were similar in placebo- and quercetin-treated mice that died after the infection. In the few infected, quercetin-treated survivors only slight changes of the renal tubular epithelials were seen.

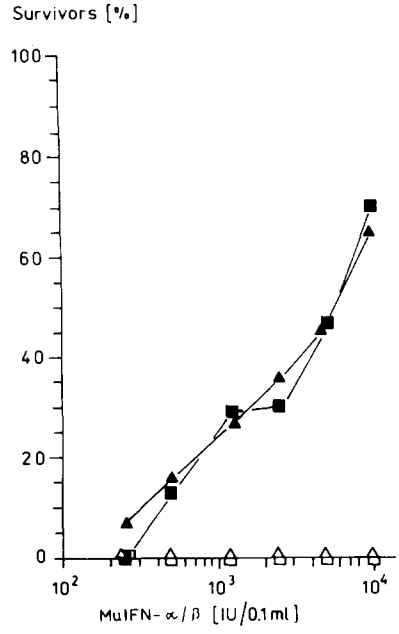


Fig. 1. Effect of different doses of MuIFN-α/β, given 1–3 h before challenge with $Mengo_M$ virus on the outcome of the infection in male $ABD2F_1$ mice. Twenty animals each in control and treatment groups were infected i.n. with 10 LD_{50} of the virus. □, i.m.; △, i.p.; placebo-treated and infected; ■, i.m.; ▲, i.p.; MuIFN-α/β-treated and infected.

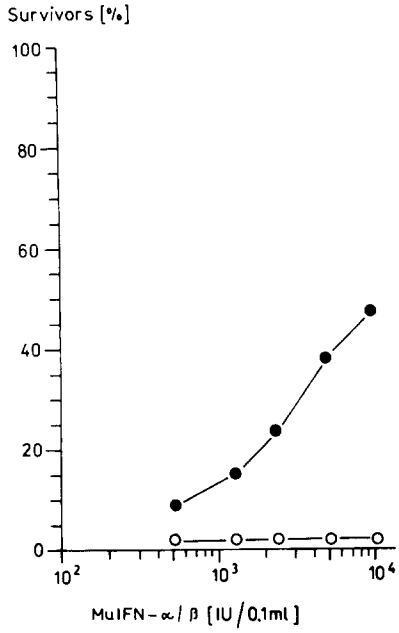


Fig. 2. Effect of different doses of MuIFN-α/β, given 1–3 h before challenge with Sindbis virus on the outcome of the infection in male $ABD2F_1$ mice. 20 animals each in control and treatment groups were infected i.n. with 10 LD_{50} of the virus. ○, i.m.; placebo-treated and infected; ●, i.m.; MuIFN-α/β-treated and infected.

Effect of MuIFN-α/β

A single protective dose of MuIFN-α/β, given 1–3 h prior to i.p. or i.n. inoculation of $Mengo_M$ virus, was effective. However, the protective efficacy was lost when treatment was delayed until 1 h after virus challenge. Similar findings have been obtained with Sindbis virus-infected mice treated in the same manner (data not shown).

TABLE 1

Incidence of histological organ lesions in $ABD2F_1$ mice surviving $Mengo_M$ virus infection following treatment with MuIFN-α/β

Organs	Dose of MuIFN-α/β per mouse[a] (IU)					
	0	500	1250	2500	5000	10000
CNS	1/4[b]	0/8	0/18	0/11	0/9	0/11
Thymus	0/4	0/8	0/18	1/11	1/9	0/11
Parotid gland	1/4	3/8	2/18	7/10	2/9	11/11
Lacrimal gland	0/4	0/8	0/16	5/10	1/9	4/5
Pancreas	1/4	0/8	0/18	0/11	0/9	2/7
Kidney	1/4	0/8	1/18	8/11	3/9	7/11

[a] Administered i.p. or i.m.

[b] Number of mice affected per number of animals investigated.

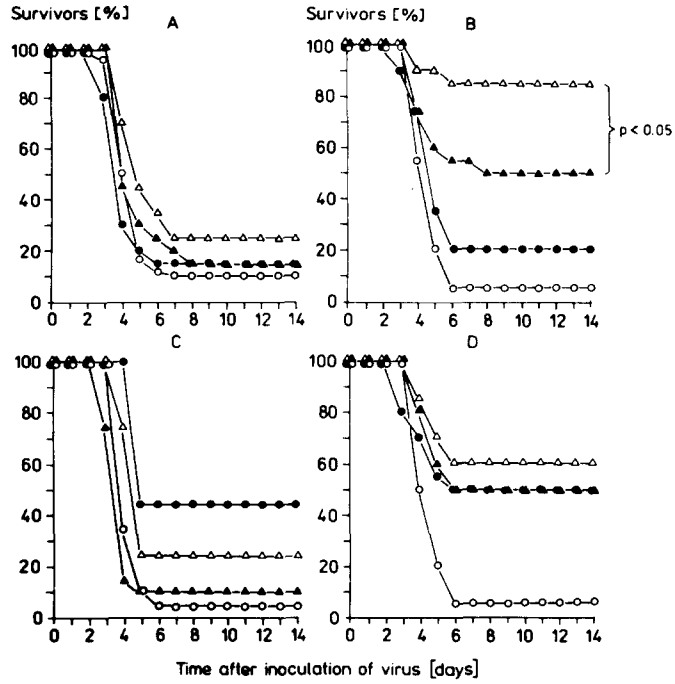
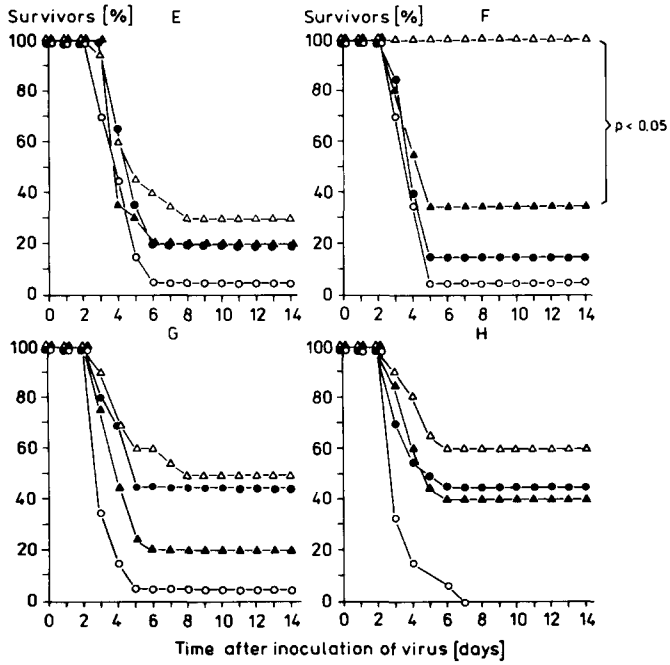


Fig. 3. For legend see facing page.

The efficacy of MuIFN-α/β on either animal model was examined by treating mice i.p. or i.m. with increasing doses 1–3 h before infection (Figs. 1 and 2). Intranasal instillation with 10 LD_{50} Mengo virus resulted in death of all animals in the mock IFN-treated groups while a single treatment with graded doses of MuIFN-α/β prevented death in a dose-dependent manner. Doses ranging from 1250–10000 IU caused a significant protective effect ($p \leq 0.05$), resulting in 30–70% survivors. The mean effective dose (ED_{50}) was found to be 5000 IU. The route of administration did not influence the antiviral effectiveness of the MuIFN-α/β preparation. Intranasal infection with 10 LD_{50} Sindbis virus killed 98% of the animals in the mock IFN-treated group. A single treatment with increasing doses of MuIFN-α/β conferred a dose-related protection. Doses of 2000–10000 IU increased the number of surviving mice from 25 to 50%. The ED_{50} proved to be as high as 10000 IU for Sindbis virus-infected mice.

Morphologically, similar acute organ lesions were found in $Mengo_M$ virus-infected, placebo-treated mice and in those animals that were not protected by the IFN-treatment. In the MuIFN-α/β-treated virus-infected mice that survived the infection the brain remained free of histological changes. However, in the other target organs slight sequels of the infection were observed, the type, intensity and localization of which proved to be independent of the dose and route of MuIFN-α/β administration (Table 1). These chronic lesions were non-specific in character and consisted of cortical atrophy of the thymus, of inflammatory reactions and epithelial vacuolization in the pancreas, the parotid and lacrimal glands, and of tubular damage of the kidneys.



Figs. 3 and 4. The relative protective effects of quercetin (oral) and MuIFN-α/β (i.p. or i.m.) in four fixed ratios of combinations against $Mengo_M$ virus infection in male $ABD2F_1$ mice. Panels A and E: Both quercetin and MuIFN-α/β were used in marginally effective treatment regimens [quercetin: 20 (initial dose) and 10 mg/kg body weight at −12, −1 and 12 h, relative to infection; MuIFN-α/β: About 500 IU 3 h prior to infection]. Panels B and F: quercetin was given at the marginally effective treatment regimen as above, and the ED_{50} of MuIFN-α/β, 5000 IU, 3 h prior to infection. Panels C and G: quercetin was given at a treatment regimen producing 50% protection [40 (initial dose) and 20 mg/kg at −12, −1, 7, 24, 32, 48 and 56 h, relative to the infection] and MuIFN-α/β at the marginally effective dose as above. Panels D and H: quercetin and MuIFN-α/β were used at the treatment regimens producing 50% protection as above. 20 animals each in the control and treatment groups were infected i.n. with 10 LD_{50} of the virus. ●, quercetin-treated and infected; ▲, (i.p., Fig. 3; i.m., Fig. 4), MuIFN-α/β-treated and infected; △, quercetin and MuIFN-α/β-treated and infected; ○, placebo-treated and infected.

Sindbis virus-infected mice, whether MuIFN-α/β or placebo-treated, that succumbed to the acute infection developed similar organ lesions.

Effect of combined treatment

Quercetin (oral) and MuIFN-α/β, given i.p. (Fig. 3) or i.m. (Fig. 4), were investigated in four fixed ratios of combinations against a lethal $Mengo_M$ virus infection in $ABD2F_1$ mice. The findings of representative experiments of each combination are shown in Figs. 3 and 4.

Combinations of the drugs at marginally effective treatment regimens had no significant protective activity against the infection (panels A and B). Likewise, no difference in the levels of protection was obtained when both drugs at treatment

TABLE 2

Effect of parenteral MuIFN-α/β and oral quercetin administered either singly or combined on $Mengo_M$ virus infection in $ABD2F_1$ mice[a]

Experiment	Drug and dose per treatment		Survivors/total	Survival (%)	Synergy[b]
	MuIFN-α/β (IU)	Quercetin (mg/kg)			
Fig. 3,	0	0	1/20	5.0	
Panel B[c]	0	20 and 10	4/20	20.0[e]	
	5000	0	20/40	50.0[d]	
	5000	20 and 10	17/20	85.0[d]	+
Fig. 4,	0	0	1/60	1.7	
Panel F[c]	0	20 and 10	9/60	15.0[e]	
	5000	0	30/60	50.0[d]	
	5000	20 and 10	56/60	93.3[d]	+

[a] Results of separate experiments with identical treatment regimens were pooled.
[b] For a definition of synergy, see Materials and Methods.
[c] For the treatment regimens, see legends to Figs. 3 and 4.
[d] Probability that the observed increase in number of survivors of the treatment group as compared with placebo-treated, infected control was due to chance (Fisher exact test): $P < 0.001$.
[e] $P > 0.05$.

schemes conferring 50% survival of mice were used in combination (panels D and H). However, synergy was observed when the ED_{50} of MuIFN-α/β was combined with quercetin at the marginally effective treatment regimen (panels B and F; Table 2). Conversely, the quercetin treatment producing 50% protection combined with MuIFN-α/β at the marginally effective dose did not increase the number of survivors (panels C and G).

In Sindbus virus-infected mice various drug combinations including the regimen resulting in synergistic effect against Mengo virus infection failed to demonstrate an enhanced antiviral efficacy (data not shown).

Discussion

Quercetin provided a macrophage-dependent protection against murine cardiovirus infections (Güttner et al., 1982; Veckenstedt and Pusztai, 1981; Veckenstedt et al., 1978, 1985a) but failed to protect Sindbis virus-infected mice. This confirms earlier observations suggesting that the antiviral activity of flavonoids in animal models is limited to picornaviruses (Ishitsuka et al., 1982; Tisdale and Selway, 1983).

Single i.p. or i.m. administration of crude MuIFN-α/β protected mice against infection with lethal doses of Mengo or Sindbis virus regardless of the route of administration, provided treatment was started prior to virus challenge. In the case of Mengo virus, this is consistent with previous studies demonstrating that IFN confers protective effect in cardiovirus-infected mice when given prior to (Weck et al., 1982) or soon after infection (Gresser et al., 1968, 1969; Heremans et al.,

1980; Olsen et al., 1976). Mengo virus infection was markedly more sensitive to MuIFN-α/β than Sindbis virus infection, as revealed by dose-response studies.

Of the various drug combinations studied, only one resulted in a higher percentage of survival than either agent alone at the dose used in the combination. Moreover, the synergistic effect was limited to Mengo virus-infected mice. Synergistic interaction between quercetin and MuIFN-α/β was noted only when quercetin administration, at a marginally effective regimen, was started prior to injection of a single protective dose of MuIFN-α/β. Thus, it appears that quercetin might have potentiating activity on MuIFN-α/β.

The available data do not enable us to explain the mechanism of action. However, the possibility should be considered that increased cyclic adenosine monophosphate production, induced by quercetin (Graziani and Chayoth, 1977), cause potentiation of MuIFN-α/β antiviral activity (Fleischmann, 1982) in Mengo virus-infected mice. Potentiation of antiviral activity of IFN by ammonium 5-tungsto-2-antimoniate or isoprinosine was also demonstrated in other cardiovirus infections of mice (Chany and Cerutti, 1977; Werner et al., 1976).

Histologic findings showed that the brain of the survivors was consistently protected from damage whereas in the other target organs only slight changes were found. These facts do not allow to conclude whether (i) the agents inhibited virus invasion into the brain or (ii) prevented damage of nerve cells by the virus once the brain infection had already become established.

Although the mechanism of the observed antiviral potentiation is not yet understood, the phenomenon is of potential significance in that flavonoids might be useful when combined with IFN in the treatment of certain picornavirus diseases.

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