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Short Communication

A note on the failure of CGP 19835 A (MTP-PE) to influence the course of influenza A2 infection in human volunteers

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Summary

A single dose of the immunomodulator CGP A (MTP-PE) given intranasally to human volunteers 24 h prior to challenge with influenza A2 virus failed to protect against infection or ameriolate any subsequent illness.

CGP 19835 A (MTP-PE); Influenza A virus; Human volunteer

The immunomodulatory molecule, CGP 19835 A (MTP-PE), the conjugate of a muramyl tripeptide (MTP) and dipalmitoylphosphatidylethanol amine (PE), has no antiviral action and does not induce interferon. However, it has been shown to reduce the severity of disease in infections of rodents (mice and guinea pigs) with a range of DNA and RNA viruses (Dietrich et al., 1988; Koff et al., 1985). It can be active when given, intranasally, as early as 3–4 weeks prior to infection with influenza virus and in doses as low as 1 μg/kg body weight (Dietrich et al., 1986).

The present study, approved by the Harrow District Ethical Committee, was undertaken to determine if CGP 19835 A, given intranasally to human volunteers prior to challenge with influenza A2 virus, would protect or modify the subsequent infection or illness.

Healthy male subjects aged 18 to 50 years were recruited and housed in isola-

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tion in groups of two or three according to our normal practice (Beare and Reed, 1977). Initial blood samples for haematological and biochemical examination were collected from all volunteers on day 0. After a 48 h quarantine period the volunteers were divided into two groups matched for age and randomly allocated to receive either drug, 0.1 ml CGP 19835 A (100 μg) into each nostril, or the same quantity of placebo. Twenty-four h later (day 3) most volunteers were challenged with an estimated $1–3 \times 10^4$ EID_{50} of A/Eng/40/83 (H_3N_2) present in allantoic fluid prepared by a single egg passage of a nasal washing containing the virus. The few remaining volunteers received saline in order to maintain the double-blind nature of the trial. Both volunteers and observer were blind to the allocation of drug or placebo and to virus or saline challenge.

Each volunteer was assessed daily and allotted a score according to the signs and symptoms present. At the end of the trial the clinical observer assessed each subject as having suffered no illness, a doubtful illness (some symptoms but not sufficiently severe or persistent to enable a firm diagnosis to be made), or a significant illness of a mild, moderate or severe nature. Nasal washings were collected prior to challenge (day 0) and on days 5 to 9 inclusive. Blood samples for repeat haematology and biochemistry were collected from all volunteers on days 3, 6 and 9 and a convalescent sample requested 2–3 weeks after challenge. Influenza virus was detected by inoculation of cultures of MDCK cells and specific antibody by a standard haemagglutination inhibition assay.

Forty-one volunteers were entered into the study but eight were excluded or failed to complete the trial for various social or medical reasons. Of the remaining 33 subjects, four were challenged with saline and 29 with influenza virus of whom 15 received CGP 19835 A and 14 placebo. One further volunteer, given drug, was excluded retrospectively as his symptoms commenced within a few hours of exposure to the challenge virus, they resembled a cold rather than experimental influenza and there was no laboratory evidence of infection with influenza A virus.

The two groups were well balanced for age and pre-challenge antibody titer (see Table 1). One moderate, four mild and two doubtful illnesses occurred in the 14 volunteers challenged with virus in the drug group compared with one moderate, three mild and three doubtful illnesses among the same number of subjects in the placebo group. The mean total clinical score for those in the drug group was 8.04 ± 11.77 and did not differ significantly from that of 5.75 ± 8.55 in the placebo group ($P = 0.22$, rank analysis of variance blocked for pre-challenge antibody titer <12, 18–24 and ≥ 36).

Virus was isolated from the post-challenge washings of five volunteers who received CGP 19835 A, all of whom excreted virus for either four or five days. Four of these subjects had symptoms and an increase in antibody titer (two $\geq$4-fold, two $\geq$2-fold). There was one sub-clinical infection in this group, a volunteer who had a total score of 1.5, excreted virus on each of four days and had a 4-fold rise in antibody titer.

There were also five volunteers in the placebo group whose post-challenge nasal washings yielded virus for between three and six days. As in the drug group, four of them had symptoms and all had an increase in antibody titer (two $\geq$ 4-fold, two

≥2-fold). There was one subject in this group who had a virologically confirmed illness from whom virus was not recovered but who demonstrated a greater than 4-fold increase in antibody concentration, 1:12 to 1:72. A further similarity to the drug group was the presence of one sub-clinical infection in a subject who had a total clinical score of 1, shed virus for four days and whose antibody titer increased from 1:36 to 1:96.

The variation in haematological and biochemical values obtained throughout the trial was similar in the drug and the placebo groups.

CGP 19835 A was well tolerated with no evidence that it induced any local or systemic adverse effect. It is equally obvious that this dose of the drug intranasally had no beneficial or deleterious effect on the volunteers' subsequent exposure to influenza A virus. Indeed, the number of significant illnesses and their severity, the number of doubtful illnesses, sub-clinical infections, virus excretors and serological responders were almost identical in the two groups. We did note a difference in the magnitude of the serological responses. Of the three subjects in each group that showed a four-fold or greater increase in antibody titer those receiving CGP 19835 A rose from <1:6 to 1:24, <1:6 to 1:24 and 1:9 to 1:36 while in the placebo group the rises were from 1:12 to 1:72, 1:24 to 1:384 and 1:18 to 1:768. The greater responses may well be the result of previous experience of a closely related virus as the pre-challenge titers were higher than those in the drug group. The greater increase could, therefore, be unrelated to any effect of MTP-PE.

It is difficult to relate our results to the finding that CGP 19835 A protects rodents against influenzal pneumonia (Dietrich et al., 1986). We gave the maximum practical dose by a route and on a time schedule that showed maximum effects in animals but this may have been inadequate. However, intranasal administration of interferon can prevent experimental influenza in man so the drug presumably

TABLE 1

Infection and illness in volunteers receiving CGP 19835 A or placebo and challenged with influenza A virus

Volunteer group and pretrial Ab titer	Number	Number with illness			Virological findings		
		Moderate	Mild	Doubtful	Virus isolated	Ab rise	Either or both
CGP 19835 A							
≤ 12	3		1	1	3	3	3
18-24	4	1	1		1		1
≥ 36	7		2	1	1		1
Total	14[a]	1	4	2	5	3	5
Placebo							
≤ 12	5		2	2	1	1	2
18-24	4	1	1	1	3	2	3
≥ 36	5				1		1
Total	14[b]	1	3	3	5	3	6

[a]14 males; mean age 29.86 ± 6.75 years.
[b]14 males; mean age 29.57 ± 7.26 years.

reached the relevant mucosal cells. As there is no biological effect of the drug, such as interferon induction that can be related to resistance in animals, it was not possible to use an indicator test for its activity in man. The protective effect in animals is lost if the dose of virus is increased; we gave 10^4 EID_{50} of a partly attenuated virus to man as this produced a relatively mild illness and does not, we believe, represent a heavy challenge. On the other hand, the disease of man was not like that in rodents; it is a mainly superficial infection in man but a pneumonia in rodents. We conclude that, although the results of this study were negative, a protective effect may be achieved by giving other doses by other routes.

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