*Antiviral Research* 14 (1990) 339–344
Elsevier



AVR 00451

# A study of the efficacy of the bradykinin antagonist, NPC 567, in rhinovirus infections in human volunteers

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(Received 29 June 1990; accepted 21 August 1990)

## Summary

In a double-blind placebo controlled trial intranasal NPC 567, a bradykinin antagonist, failed to alleviate the symptoms of experimental rhinovirus colds. Indeed, there was evidence that the drug enhanced the symptoms although no irritant effect was detected on the uninfected nasal mucosa.

Rhinovirus; Bradykinin antagonist (NPC 567); Human volunteer

## Introduction

Attempts to demonstrate the presence of certain mediators of tissue reaction to infection, e.g. histamine and leukotrienes, in the nasal secretions of individuals with colds have proved negative or, at best, equivocal (Egglestone et al., 1984; Naclerio et al., 1987; Callow et al., 1988; Proud et al., 1990). However, kinins, including bradykinin, have been shown to be generated during the coryzal phase of both experimental (Naclerio et al., 1987) and natural (Proud et al., 1990) rhinovirus infections and the intranasal administration of bradykinin produces symptoms indistinguishable from those of a rhinovirus cold (Proud et al., 1988).

NPC 567 is a bradykinin antagonist [$DArg^0$, $Hyp^3$, $DPhe^7$] that inhibits the response to bradykinin in vitro by competitive blockade of the kinin receptor. Under these conditions NPC 567 has 10% of the binding activity of bradykinin (Burch et al., 1990). The efficacy of intranasal NPC 567 in rhinovirus induced colds in human subjects has, therefore, been investigated. Studies in which treatment began before a cold were initiated elsewhere, so in this study treatment began when

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cold symptoms had just appeared, the timing which would be most suitable for application to clinical practice.

## Materials and Methods

### *Trial design*

The trial, approved by the Harrow and District Ethical Committee at Northwick Park, was conducted in accordance with our standard procedure for double-blind placebo-controlled trials (Beare and Reed, 1977) which permits a daily assessment of colds by means of a clinical score and nasal secretion weight in addition to an overall evaluation of the colds. In brief, after a 48-h quarantine period, healthy volunteers were challenged with rhinovirus type 2 and, 1 h later, with rhinovirus strain EL. Those who developed colds between 36 and 84 h after challenge, self-administered placebo or NPC 567 (2.5 mg/ml), 0.2 ml to each nostril, six times a day until the end of the trial, a maximum of five days. A member of staff supervised the administration. The spray contained sodium chloride 0.6% (w/v) citric acid 0.022% sodium citrate 0.7% in distilled water and was preserved with methyl paraben 0.18% and propyl paraben 0.2%; the placebo consisted of the vehicle without the drug. Randomisation was only partial in those trials which included females in order to ensure that those at risk of pregnancy would receive only placebo. Volunteers remained in isolation throughout and were assessed clinically every day. Nasal washings for virus isolation were collected daily commencing 36 h after virus challenge. Blood samples for haematological and biochemical tests were collected from all volunteers before entry into the trial and again, from those who had received medication, at the end of the trial. In order to evaluate any transient relief of symptoms that might result from medication, volunteers were asked to assess the severity of their own colds as well as the four symptoms: blocked nose, runny nose, sore throat and cough, on an analogue 10 cm scale graduated from 0 to 100. Assessments were made immediately before and again one hour after the 11 am dose of medication each day; the second assessment was made on an identical scale adjacent to the first.

## Results

Forty-five subjects took part in one or other of two double-blind, placebo-controlled tolerance studies conducted by the Nova Pharmaceutical Corporation (unpublished data). Medication consisted of 36 doses of 0.4 ml of NPC 567 (2.5 mg/ml) or placebo intranasally over six days and both were well tolerated. A further 10 volunteers took part in a similar tolerance study of 30 doses of medication at the CCU. One volunteer (placebo) was withdrawn from the study because of epistaxis. The total clinical score of each of the remaining subjects was nil (unpublished data).

During the course of six trials 80 volunteers were allotted to the efficacy study. Females were included only in the last two trials after regulatory permission was

TABLE 1

| Group and composition | Mean age | Psychological scores | Colds | Mean total clinical score | Mean total nasal secretion weight |
|---|---|---|---|---|---|
| Drug<br>9 males<br>2 females | 34.82 ± 7.60 yrs | I/E 10.00<br>Obs. 2.00 | 3 moderate<br>8 mild | 29.86 ± 20.25 | 37.07 ±45.70g |
| Placebo<br>7 males<br>4 females | 34.91 ± 8.92 yrs | I/E 9.09<br>Obs. 3.09 | 11 mild | 19.86 ± 11.68 | 19.54 ± 25.60g |

I/E, introversion/extroversion; Obs., obsession.

received and numbered 22. Six subjects were excluded, two because of wild colds, two as contacts of wild colds, one because of abnormal biochemical test results and one withdrew for domestic reasons. Of the remaining 74 volunteers, 22 were diagnosed as suffering a cold at the requisite time and were given medication; 11 received NPC 567 and 11 placebo. The two groups were well balanced for sex and age (see Table 1). Three of the 11 colds in the drug group were classed as moderate and the remaining eight as mild compared with all 11 colds in the placebo group being graded as mild. The mean total clinical score and mean total nasal secretion weight of the drug group were 29.86 ± 20.25 and 37.07 ± 45.70 g respectively compared with 19.86 ± 11.86 and 19.54 ± 25.60 g in the placebo group. These differences do not reach statistical significance at the $P$=0.05 level. There was no evidence of an effect when men and infertile females were analysed separately (data not shown).

A more accurate appraisal of the effect of NPC 567 on the course of a rhinovirus cold is obtained by comparing the mean daily clinical score and the mean daily nasal secretion weight of the two groups from the onset of the colds (see figure). The mean daily clinical score and mean daily nasal secretion weight were very similar in the two groups on the first day but thereafter the values for the drug group were always greater than those of the placebo group; this was most marked for the mean daily nasal secretion weight but the only difference to reach statistical significance was that for nasal secretion on day 6 ($0.05>P>0.01$).

These two parameters are not entirely independent as the number of tissues used, which reflects the amount of nasal secretion, contributes significantly to the clinical score. If the mean daily clinical scores are re-calculated excluding the values attributable to the tissue count (i.e. an evaluation of the cold symptoms other than a runny nose) then the patterns of the two groups becomes much closer and, indeed, are reversed on day 6 (see figure).

In the volunteers' analogue assessment of their own colds changes of ± 5 mm or less were ignored. A change in the severity of their colds and symptoms was recorded in 76 of 188 (40.4%) assessments made by NPC 567 recipients compared with 78 of 193 (40.4%) assessments made by those given placebo. In the drug group improvement was recorded in 51 (67%) instances and an increase in severity in 25 (33%). The corresponding figures for the placebo group were 61 (78%) and 17 (22%) respectively. Changes in excess of ± 25 mm indicating a substantial change in severity, occurred in 4 of the 188 (2%) assessments in the NPC 567 group

and in 3 of these the symptoms were considered to have increased in severity. Such changes were more common in the placebo group with 21 instances among the 193 (11%) assessments and all but one signified a beneficial effect. There is little evidence that NPC 567 produced a consistent short term effect in any volunteer, often an improvement on one day being counterbalanced by deterioration on another. Placebo had a more consistent effect and was generally beneficial. However, it is interesting to note that 13 of the 21 substantial changes occurring among placebo recipients were reported by just two subjects and probably relates more to the personality of the volunteer than the medication. Similarly these data fail to support the hypothesis that it is important at which stage of a cold the medication is given.

The variation in haematological and biochemical values obtained before and after medication was similar in the drug and placebo recipients.

## Discussion

When medication commenced the two groups were well matched and the severity of the colds were similar. It was not possible to make a formal power calculation

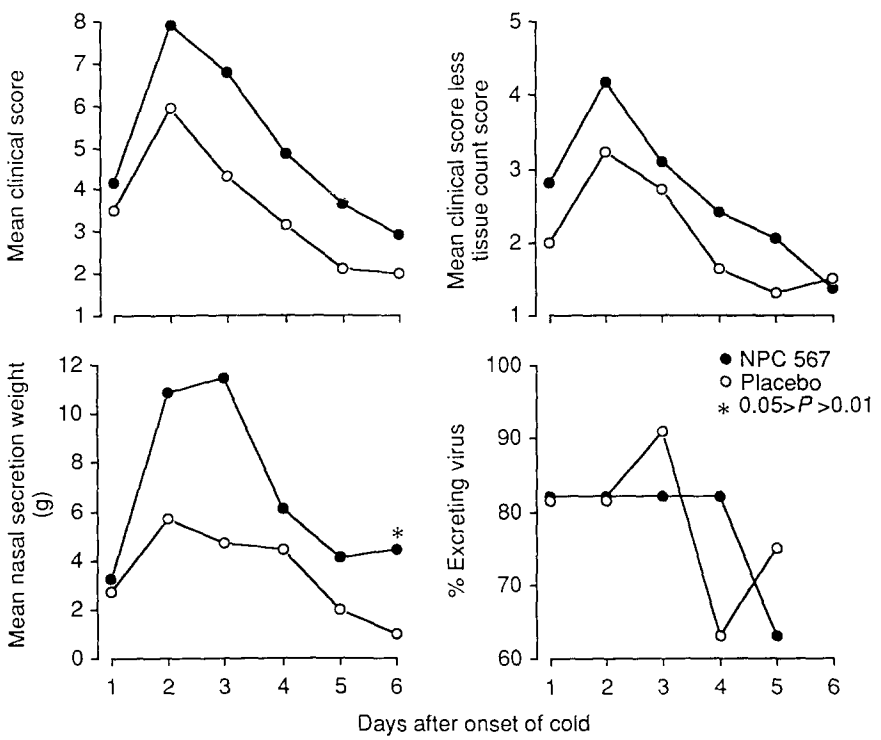


Fig. 1. Results of daily assessment of volunteers given the bradykinin antagonist NPC 567 or placebo from the first day of a rhinovirus common cold.

for this study, but for earlier prophylactic studies of a similar size it was estimated that a 50% reduction in symptoms could be detected in 90% of trials with $P<0.05$. It must be realised that the clinical scores were allotted to subjects between 9 and 10 am each day and reflect the clinical condition at that time and the symptoms experienced and tissues used during the previous 24 hours. Therefore, the majority or all of the clinical score and nasal secretion weight on day 1 has been acquired before medication commences. After medication had started the colds in those receiving drug were more severe than those in the placebo group although the differences were statistically significant on only one occasion – the mean nasal secretion weight on day 6. The greater severity of the colds in the drug group can be attributed to increased nasal secretion as can be seen when the contribution of the tissue count is deducted from the clinical score (see Fig. 1). Furthermore, the volunteers' own assessment showed no evidence of a specific short termed beneficial effect of NPC 567 although there was a placebo effect. Approximately 70% of the self-assessed changes were considered to be beneficial in both groups. However, of the substantial changes, $>\pm 25$, three of the four in the drug group were adverse compared with 20 of the 21 similar changes in the placebo group which were beneficial.

There is no evidence that NPC 567 has a beneficial effect on the course of rhinovirus induced colds. Indeed, from the clinical observations and the volunteers' self-assessments it would appear that the drug acts as a mild irritant despite the evidence obtained in the tolerance studies. The irritant effect must be attributed to the drug itself as the placebo contained all the ingredients of the vehicle. However, it has been observed previously that antivirals that are innocuous to a normal nasal epithelium can be irritant to an already inflamed mucosa (Al-Nakib et al., 1989).

There are several possible reasons for our failure to demonstrate a beneficial effect of NPC 567 in man. The dosage may have been insufficient or the drug may have failed to reach the kinin receptor. NPC 567 inhibits the binding of [$H^3$]-bradykinin to neuroblastoma cells with a binding constant (Ki) at $4.0 \pm 0.2$ nM and to guinea pig ileum membrane with Ki $36.1 \pm 0.6$ nM. Concentrations of $10^{-5}$ M have been shown to inhibit bradykinin-mediated effects in animal models (unpublished). NPC 567 has only 10% of the binding capacity of bradykinin to which the receptors would already have been exposed when a cold becomes clinically apparent and so place the drug at a distinct competitive disadvantage. It might be inhibited by some component of nasal secretion but no biological inhibitors are known at the moment. Lastly it is possible the bradykinins do not play a dominant role in the coryza of rhinovirus induced colds.

## Acknowledgements

We wish to acknowledge the willing co-operation of the volunteers, the work of the nursing sisters in caring for them and the laboratory staff of the Common Cold Unit for the virological examination of specimens. We thank Dr W. Boggs of Nova Pharmaceutical Corporation for providing the drug and the information.

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