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A study of the efficacy of the immunomodulatory compound 7-thia-8-oxoguanosine in coronavirus 229E infections in human volunteers

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Summary

Intranasal 7-thia-8-oxoguanosine (NARI 10146) compared with placebo had no influence on the course of experimental coronavirus 229E infections in human volunteers. Possible reasons are discussed for the failure to confirm successful rodent experiments in man.

Introduction

NARI 10146, recently described by Nagahara *et al.* (1990), is a nucleoside analogue, 7-thia-8-oxoguanosine and, like a number of other 8- or 7- and 8-substituted guanosines, has been shown to possess immunomodulatory activity (Goodman & Hennen 1986; Wicker *et al.* 1987; Dorsch *et al.*, 1988; Koo *et al.*, 1988).

In vitro and *in vivo* studies in rodents (mice) have shown that NARI 10146 stimulates the proliferation of B lymphocytes, natural killer (NK) cells, macrophages, cytotoxic T cells, the expression of surface antigens, immunoglobulin secretion by B lymphocytes, the production of interleukin-3, interferon and enhances antibody-dependent cellular cytotoxicity. Experiments on human peripheral blood lymphocytes confirmed the stimulation of NK cells, cytotoxic T cells, the expression of surface antigens, and demonstrated enhanced production of interleukin-1 (Nagahara *et al.*, 1990; Jin *et al.*, 1990; Ojo-Amaize *et al.*, 1991).

These immunomodulatory activities of NARI 10146 are considered to be responsible for the protective effect observed against a range of virus infections in rodents. Most satisfactory results were obtained when the drug was administered prior to, or shortly after, exposure to virus and when given as a divided, rather than a single, dose (Smee *et al.*, 1989, 1990a,b). The model infections in which a response to both intraperitoneal and intranasal NARI 10146 was seen included one in which a coronavirus produced pneumonia in rats. NARI 10146 also reduced the mortality and lengthened the survival time of the mice that succumbed to an encephalitis produced by the intracranial inoculation of a human respiratory coronavirus.

These observations in rodents have led to the present study of the efficacy of NARI 10146 in infections with coronavirus 229E in human volunteers.

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Results

Forty-eight volunteers were used in the study. Six subjects were excluded, two because of wild colds, two as contacts of wild colds, one because of hypertension and one because of abnormal results of biochemical tests. An additional two subjects were excluded, one for an allergic reaction originally believed to be a wild cold, and another as a contact of the supposed wild cold. One volunteer withdrew from the study for domestic reasons.

Of the remaining 39 volunteers, one was challenged with saline and 38 with virus, of whom 18 received placebo and 20 NARI 10146. As it was necessary to ensure that those at risk of pregnancy received placebo, the two groups were not ideally balanced for sex; there were no males in the placebo group compared with eight (40%) in the drug group. The two groups were also unbalanced for age and pretrial antibody titre; the mean age of the placebo group was 35.61 ± 9.95 years compared with 41.30 ± 9.18 years for the drug group and 50% of the drug group had high pretrial antibody titres compared with only 22% in the placebo group (Table 1).

There were 12 significant colds (60%) among the 20 subjects receiving drug, one moderate and 11 mild, while 11 colds (61%), all mild, occurred in those given placebo. There was laboratory evidence of virus infection in all individuals who developed colds. Clinical scores were calculated from daily records of symptoms, signs and counts of the number of paper handkerchiefs used (Beare

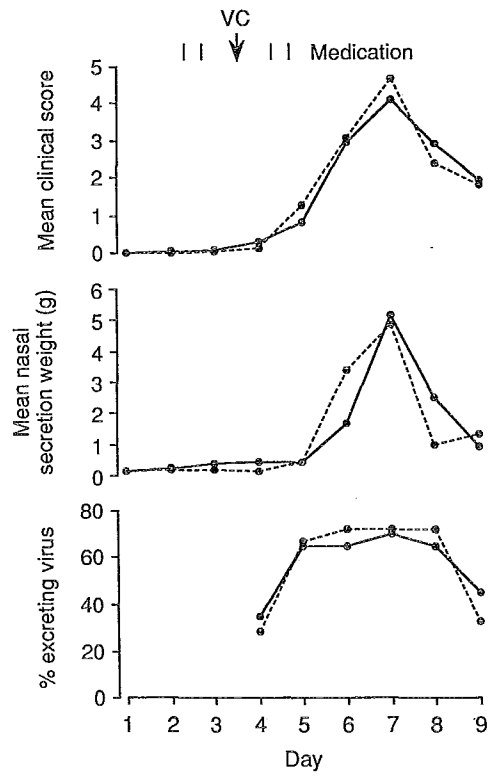

Fig. 1. Daily record of clinical status and virus excretion in groups of active- and placebo-treated volunteers. None of the difference are statistically significant (●-----●) Treated; (●——●) placebo.

and Reed, 1977). The mean total clinical scores for the two groups were very similar, 13.13 ± 12.15 in the drug group and 13.39 ± 12.83 in the placebo group, as were the mean total nasal secretion weights, 11.10 ± 18.86 g and 11.15 ± 16.50 g, respectively. The mean daily clinical score and mean daily nasal secretion weights for the two groups were almost identical (Fig. 1). Sixteen of the 20 volunteers (80%) given drug and virus showed laboratory evidence of infection, i.e. virus isolation and/or a significant rise in antibody titre, compared with 14 of 18 subjects (78%) receiving placebo and virus. There was no delay in virus shedding in the treated group (Fig. 1). No difference in any of these parameters reached statistical significance at the $P = 0{\cdot}05$ level. The variation in haematological and biochemical values obtained before and after medication was similar in the drug and placebo groups.

Discussion

It is unfortunate that the two groups were unbalanced for sex, age and pre-trial antibody titre, but all of these resulted mainly from ensuring that those at risk of pregnancy, predominately younger females, did not receive 7-thia-8-oxoguanosine. In each instance the imbalance would be expected to favour more frequent infection and a greater incidence and severity of colds in the placebo group than in the drug group. This could have resulted in an apparent protective effect by the drug but in fact the frequency of infection (80% versus 78%) and colds (60% versus 61%), the severity of illness as measured by the mean total clinical score (13.13 versus 13.39) and the mean total nasal secretion weight (11.10 g versus 11.15 g) in the drug and placebo group, respectively, show that the two groups reacted to challenge with coronavirus 229E in an almost identical manner.

We think there are three possible reasons for our failure to demonstrate a protective effect of 7-thia-8-oxoguanosine in man comparable with that observed in rodents. Firstly, the dosage could well have been inadequate: the total amount of 7-thia-8-oxoguanosine given intranasally to volunteers was 16 mg; the equivalent of 0.25 mg kilo $^{-1}$ for a 70 kg subject, whereas in rodents 50 mg/kilo^{-1} given intraperitoneally were necessary to demonstrate protection against the rat coronavirus. Although, because the drug was given locally to man, less might be required, the amount given may still have been insufficient.

Table 1. Clinical and laboratory responses of treated and untreated volunteers.

Group	Pretrial antibody titre[a]	No.	Colds: Significant: Moderate	Colds: Significant: Mild	Colds: Not significant: Doubtful	Laboratory evidence of infection
NARI 10146	<3.52	4		4	0	4
	3.53 – 3.68	6	1	4	0	5
	>3.68	10		3	3	7
	Total	20[c]	1	11	3	16
Placebo	<3.52	8		8	0	8
	3.53 – 3.68	6		3	0	5
	>3.68	4		0	0	1
	Total	18[c]		11	0	14

a. $\log_{10}$ arbitrary units determined by an ELISA test.
b. Virus isolation or a significant rise in antibody.
c. 8 male, 12 females. Mean age 41.30 ± 9.18 years.
d. 18 females. Mean age 35.61 ± 9.95 years.

Secondly, the immune system of man may react to the drug in a different manner to that of rodents. There is evidence that its protective effect in some virus-infected rodents is the result of increased interferon production (D. F. Smee, H. A. Alaghamandan, A. Jin and B. S. Sharma, manuscript submitted for publication). It is known that specific interferon inducers, e.g. double-stranded RNA and poly I:C, which are very efficient in rodents, provoke a much smaller response in man (e.g. Aoki *et al.* 1978).

Thirdly, in rodents the efficacy of NARI 10146 varies with the size of the virus challenge, being more efficient when a small dose of virus is employed (Smee *et al.*, 1989). This may well apply to man also, and although $100 TCID_{50}$ is a relatively small infectious dose for man it may be sufficiently large to obscure a possible beneficial effect.

These results with 7-thia-8-oxoguanosine are essentially the same as those observed with another immunomodulatory compound, MTP-PE (Higgins *et al.*, 1989), and they raise precisely the same questions concerning dosage, size of virus challenge and species reaction. The search for drugs which enhance the immune response should be continued as they are likely to have a beneficial influence on a wider range of infections than synthetic antiviral compounds whose activity would be expected to be restricted to a single group of agents. However, before further volunteer studies are undertaken some measurable biological effect in man, preferably one related to protection against infection, such as enhanced interferon or local specific IgA production, must be observed so that the dosage can be logically selected.

Materials and Experimental procedures

An independent tolerance study in man showed that intranasal NARI 10146 was non-irritant and produced no increase in nasal secretion (ICN unpublished data on file).

The trial, approved by the Harrow and District Ethical Committee at Northwick Park Hospital, 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, healthy volunteers in isolation were given placebo or 4 mg NARI 10146, intranasally, 24 and 12 h before and after intranasal challenge with nasal drops containing an estimated $100 TCID_{50}$ coronavirus 229E. The placebo was 3% w/v sodium bicarbonate and the active treatment contained $10 mg ml^{-1}$ of drug. Both were given as a nasal spray delivering two puffs of 0·2 ml. Volunteers were assessed clinically each day and nasal washings for virus isolation were collected daily commencing 48 h after virus challenge. Blood samples for haematological and biochemical tests were collected before entry into the trial and again 5 days after the initial medication. A convalescent sample of blood was taken 10 days after the end of the trial. Specific antibodies were assayed by ELISA. Previous work indicates that the presence of high titre antibody correlates with resistance to infection (Callow, 1985). Differences in the frequency of colds and antibody rises between the drug- and placebo-treated groups were tested for significance using the chi-squared test with the correction of Yates. Clinical scores and nasal secretion weights were tested by rank analysis of variance in which the data were blocked into three strata according to prechallenge antibody titre of each volunteer, <3.52, 3.53–3.68 and >3.68 log_{10} arbitrary units.

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