Cimetidine as modulator of the cell-mediated immune response *in vivo* using the tuberculin skin test as parameter

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The *in vivo* pharmacological effects of cimetidine on delayed hypersensitivity reactions in healthy volunteers were studied using the tuberculin skin test as parameter. Cimetidine clearly enhanced this reaction when it was administered well before antigenic stimulation.

Keywords cimetidine immune modulation skin test *in vivo*

Introduction

Histamine is known to down regulate the immune response after antigenic stimulation (Rocklin & Beer, 1983). With the delayed hypersensitivity reactions this is partially caused by stimulation of the histamine H₂-receptors on T-suppressor (Ts) and cytolytic-T(Tc)-cells (Melmon *et al.*, 1981; Rocklin & Beer, 1983). The normal lytic function of Tc-cells is suppressed while the Ts-cells are stimulated to release the 'histamine induced suppressor factor' (HSF) which inhibits other cell functions, i.e. T-helper cell proliferation (Rocklin & Beer, 1983). The production of HSF is abrogated by culture in medium containing a histamine H₂-receptor blocker but not by the addition of a histamine H₁-receptor blocker (Rocklin & Beer, 1983).

Cimetidine, a histamine H₂-receptor antagonist inhibits the immune suppression mediated by Ts-cells (Sahasrabudhe *et al.*, 1987). Although cimetidine is well documented to be a modulator of immune reactions in animal studies (Gifford *et al.*, 1981; Hansbrough *et al.*, 1986), only a few *in vivo* clinical trials have been done (Jorizzo *et al.*, 1980; Tonnesen *et al.*, 1988; White & Ballow, 1985). Most of the clinical studies have been done on relatively small immune compromised patient populations.

The aim of the present study was firstly to verify the immune enhancing effect of cimetidine in healthy individuals and secondly to correlate, *in vivo*, the time sequence of antigenic stimulation to this immune modulatory effect using a recognized cell mediated immune reaction i.e. the tuberculin reaction as parameter.

Methods

One hundred volunteers were randomly allocated to treatment in this double-blind, placebo-controlled trial over 15 days. Strict inclusion criteria were applied and only healthy students with similar socio-economic backgrounds who were on no medication, between 18 and 25 years of age and who had previously received BCG inoculation were admitted to the trial after ethical approval and informed consent had been obtained.

The tuberculin skin test (Mantoux) is a reliable test frequently used to determine a patient's cellular immune response to the tubercle bacilli (Hale, 1985; Snider, 1985). The test was administered by injecting 0.1 ml of Japan-freeze-dried tuberculin purified protein derivative (PPD) (Japan BCG-Laboratory, Tokyo) intradermally on the volare aspect of the volunteer's forearm. The injection consisted of 5 TU-PPD in tween-80 as antigen (Hale, 1985) and was administered on two occasions, firstly on the left forearm (day 5) and after a 7 day interval (i.e. day 12) on the right arm. The test results were interpreted 72 h after administration by one examiner. The size of induration was measured transverse to the long axis of the forearm (Hale, 1985; Snider, 1985).

A 300 mg oral dose of cimetidine (Tagamet[®] Smith Kline and French (Pty) Ltd) taken 4 times a day gives plasma concentrations in humans comparable with those reached during animal trials (Gifford *et al.*, 1981). In order to achieve at least these plasma levels a dose of 400 mg cimetidine 4 times a day was decided upon. This was in

Table 1 The treatments the groups received during the study

	Day														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Group 1 (<i>n</i> = 13)	P	P	P	P	P T:L	P	P	P E	P	P	P	P T:R	P	P	— E
Group 2 (<i>n</i> = 12)	C	C	C	C	C T:L	C	C	P E	P	P	P	P T:R	P	P	— E
Group 3 (<i>n</i> = 13)	P	P	P	P	P T:L	P	P	C E	C	C	C	C T:R	C	C	— E
Group 4 (<i>n</i> = 9)	C	C	C	P	P T:L	P	P	P E	P	P	P	P T:R	P	P	— E
Group 5 (<i>n</i> = 12)	P	P	P	P	C T:L	C	C	P E	P	P	P	P T:R	P	P	— E

Cimetidine C; Placebo P; Evaluation of induration E; Tuberculin test T; Left fore-arm L; Right fore-arm R.

The tuberculin reaction was interpreted 72 h after administration.

accordance with the dosages used in earlier studies (Wolfe *et al.*, 1979) as well as those frequently used in gastric diseases (Meuwissen & Klinkenberg-Knol, 1988).

Five treatment modalities were compared (see Table 1 for detail). Group 1, the placebo control, served as a detector for the booster phenomenon (Thompson *et al.*, 1979) caused by serial tuberculin testing. Any detected booster effect could then be brought into consideration in the statistical analysis. To minimize further this effect, the two tuberculin tests were administered within 7 days of each other. As this allowed no washout period after placebo, the active treatment groups (groups 2 and 3) were duplicated.

Group 4 was designed to detect any carry-over effect after cimetidine was discontinued for 2 days.

Group 5 was designed to determine whether the timing of antigenic stimulation and treatment had any effect on the outcome of the tuberculin test. Treatment commenced on the day of the first tuberculin test.

As the size of the tuberculin reaction varies from volunteer to volunteer it is appropriate to look only at differences in reactions. Statistical analysis was done firstly by evaluating the mean difference in reaction between the first and second reaction within each group and secondly by comparing this mean difference to that achieved during placebo administration (group 1).

Results

During the trial one volunteer suffered slight epigastric discomfort on active treatment. There were no sex differences regarding allocation of, or response to, treatment. Three volunteers were dropped owing to poor compliance. All volunteers who did not respond to Mantoux testing were considered non-responders and were omitted from statistical analysis (Avella *et al.*, 1978).

The non-responder drop-out rate was high (*n* = 38). This is in accordance with results obtained with tuberculin testing in this specific population group in South Africa (Fourie & Kimbel, 1987).

The placebo response of nine volunteers was clinically insignificant (< 10 mm) but became significant (> 10 mm) (Snider, 1985) after cimetidine.

The control group (Group 1) demonstrated no booster phenomenon and there was no significant change in tuberculin reaction after both tests (Table 2).

After receiving cimetidine treatment, both groups 2 and 3 showed a significant increase in tuberculin reaction within the group and also when compared with the placebo control group (Table 2).

Contrary to White & Ballow (1985) who reported a positive carry-over effect on plasma immunoglobulin levels we were not able to demonstrate any carry-over effect on the

Table 2 Mean size of induration (mm) $\pm$ s.d.

Group	1st test	2nd test	Mean absolute difference
1. ($n = 13$)	13.3 $\pm$ 4.3	13.4 $\pm$ 3.8	0.1 $\pm$ 2.3
2. ($n = 12$)	13.7 $\pm$ 6.4	11.3 $\pm$ 4.7	*2.4 $\pm$ 4.1
3. ($n = 13$)	10.8 $\pm$ 3.6	16.0 $\pm$ 5.4	**5.2 $\pm$ 6.2
4. ($n = 9$)	12.2 $\pm$ 3.9	13.8 $\pm$ 2.8	1.6 $\pm$ 2.4
5. ($n = 12$)	11.9 $\pm$ 6.2	11.6 $\pm$ 5.6	0.3 $\pm$ 5.6

Statistically significant * ($P < 0.05$) and a statistically highly significant ** ($P < 0.01$) increase in tuberculin reaction after cimetidine treatment.

The paired *t*- and the nonparametric signed ranks tests were used.

cutaneous delayed hypersensitivity reaction in healthy volunteers 36 h after cessation of cimetidine (Group 4).

The reason for the insignificant effect observed in group 5 is unclear but may be due to inter-individual variation.

Discussion

The immune response is controlled by various complex subsets of immune competent cells responding differently to various stimuli. If the cell surface receptors were distributed randomly on these cells, the immune response would have been chaotic. Various experiments have demonstrated a non-random distribution of

receptors explaining a specific response to certain autacoids (Khan *et al.*, 1985; Melmon *et al.*, 1981; Rocklin & Beer, 1983). It is thus possible for a histamine H₂-receptor blocker to modulate T-suppressor cell function selectively.

This study has shown clear evidence that a therapeutic dosage regime of cimetidine, enhances the delayed type hypersensitivity reaction in healthy volunteers. This is contrary to previous findings (Avella *et al.*, 1978; Wolfe *et al.*, 1979).

However, analysis of the data of Wolfe *et al.* (1979) shows an insignificant enhanced response after cimetidine pretreatment. Avella *et al.* (1978) reported a significant increase in the delayed cutaneous hypersensitivity reaction using streptokinase-streptodornase as antigen. They were unable to show *in vivo* augmentation of the tuberculin reaction, possibly owing to trial design and a small trial size.

Consistent with other authors (Hansbrough *et al.*, 1986) we found that cimetidine had to be administered well in advance of antigenic stimulation to have any clinically significant effect. It would appear that antigenic stimulation followed by re-stimulation while on cimetidine gives rise to a more prominent immunomodulatory effect (group 2 vs 3).

These results indicate a positive effect on immune response by cimetidine in healthy volunteers. Similar results (Tonnesen *et al.*, 1988) have previously been reported in cancer patients. Further investigation of these drugs in comprehensive controlled clinical trials is indicated.

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