

# Mitogen stimulation of peripheral blood lymphocytes of duodenal ulcer patients during treatment with cimetidine or ranitidine

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**SUMMARY** During a double-blind randomised clinical trial of cimetidine and ranitidine in the management of duodenal ulcer, the response of patients' peripheral blood lymphocytes to optimal mitogenic stimulation *in vitro* has been measured. Treatment with cimetidine, but not ranitidine, was associated with a significant increase in the proportion of peripheral blood lymphocytes responding to this optimal mitogenic stimulation. We conclude that these effects of cimetidine may not be mediated at classical histamine H<sub>2</sub>-receptors.

Exogenous histamine in non-toxic concentrations suppresses proliferation *in vitro* of mitogen-stimulated lymphocytes.<sup>1</sup> It has been proposed that the suppressive activity is mediated through H<sub>2</sub>-receptors, as it is inhibited by H<sub>2</sub>-antagonists<sup>2</sup> and potentiated by H<sub>2</sub>-agonists.<sup>3</sup> Histamine appears to stimulate T-cells to release a suppressive factor,<sup>4</sup> but a small number of adherent cells are necessary.<sup>5</sup> Studies with human lymphocytes have shown that concanavalin A (Con A) activates suppressor cells by provoking histamine release and that this effect is blocked by cimetidine (an H<sub>2</sub>-antagonist).<sup>6</sup> moreover, cimetidine augments the proliferative response of human lymphocytes to both mitogens and antigens.<sup>7</sup> Ranitidine behaves as a specific H<sub>2</sub>-antagonist to conventional models (such as inhibition of gastric secretion)<sup>8</sup> with a molar potency four to eight times greater than cimetidine in man<sup>9-11</sup> but it lacks the imidazole nucleus common to histamine and cimetidine (Figure).

This study was undertaken to determine whether cimetidine and ranitidine, when used in the treatment of patients with clinically active duodenal ulcer, would significantly alter the function of peripheral blood lymphocytes in these patients. The model of lymphocyte function chosen for study was the *in-vitro* response to mitogenic stimulation with phytohaemagglutinin Con A, and pokeweed mitogen.

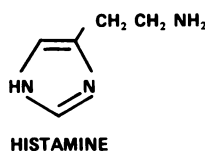
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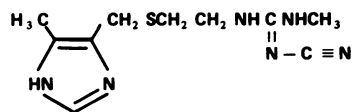
## Methods

### PATIENTS

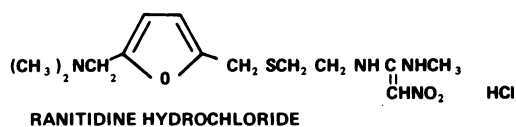
Patients gave informed consent to the study which had the approval of the Dundee District Ethical Committee. The 33 patients studied had endo-



HISTAMINE



CIMETIDINE



RANITIDINE HYDROCHLORIDE

Figure Chemical structures of histamine, cimetidine, and ranitidine hydrochloride

scopically proven duodenal ulcers and were participating in a double-blind randomised clinical trial which has been reported in detail elsewhere.<sup>12</sup> After randomisation patients were treated with cimetidine 800 mg/day or ranitidine 320 mg/day, the dosing schedule being one tablet with breakfast and lunch and two before retiring. The patients in the two treatment groups were comparable (Table 1). After 28 days of treatment, eight of 18 patients given cimetidine and 11 of 15 given ranitidine had achieved complete healing of their ulcers, while 10 of 18 and 11 of 15 respectively were free of symptoms.

#### INVESTIGATIONS

Where possible, each patient was studied on three occasions – namely, before treatment, after 14 days of treatment (one to two hours after dosing) and after 28 days of treatment (14–18 hours after the last tablet had been taken). Certain blood samples were unsuitable for culture – namely, in the cimetidine series, those from three patients at 14 days and from four patients at 28 days and in the ranitidine series, one each at the pretreatment, 14 days' and 28 days' sampling times. No patient was included in the series if more than one sample were missed.

Blood was withdrawn from the patient who had been fasted overnight and after at least 15 minutes' rest so that peripheral blood lymphocytes in the sample would be representative of the basal state.<sup>13</sup> Blood for mitogen stimulation tests was taken into heparinised tubes while blood for counting the total numbers of lymphocytes was taken into sequestrene tubes.

#### LYMPHOCYTE STIMULATION TESTS

The laboratory workers in the group did not know which drugs individual patients were receiving nor the clinical progress when the tests were being performed. Mononuclear cells were isolated from heparinised blood samples by Ficoll/Hypaque density gradient centrifugation.<sup>14</sup> Lymphocytes were the predominant cell type in the specimens used in this investigation (>90%); as monocytes are necessary for the response to mitogen stimulation<sup>15</sup> no attempt was made to purify the lymphocyte suspension by removal of the phagocytic cells lest

this procedure introduced an uncontrollable variable between samples from different patients. The harvested cells were washed in tissue culture fluid before testing.

The method used for study of lymphocyte response to mitogen stimulation has been described in detail previously.<sup>16</sup> Briefly, the incubation mixture in each round-bottomed well of a microtitre plate (Sterilin Ltd, Teddington, England) contained (1) 100  $\mu$ l lymphocyte-rich suspension ( $2 \times 10^5$  cells), (2) 50  $\mu$ l mitogen dissolved in culture fluid, and (3) 20  $\mu$ l autologous plasma. The plates were sealed with adhesive tape (Flow Laboratories, Irvine, Scotland) and incubated at 37°C for 22–24 hours. The standard tissue culture fluid was medium TC199 (Gibco-Biocult, Paisley, Scotland) supplemented with L-glutamine (200 mM), penicillin (200 IU/ml) and streptomycin (100  $\mu$ g/ml). The mitogens (used at optimal concentrations) were phytohaemagglutinin (Wellcome Reagents Ltd, Beckenham, England) at 5.0  $\mu$ l HA15/ml TC199, Con A (Sigma, St Louis, USA) at 100  $\mu$ l/ml, and pokeweed mitogen (Gibco-Biocult) at 20  $\mu$ l/ml. The exact duration of culture was noted at the time of harvesting when the cells were disaggregated and dispersed by repeated pasteur pipette mixing. The relative numbers of lymphocytes and phagocytic cells in the cells harvested from the control wells were determined by volume spectroscopy<sup>17</sup> – with the methods currently used in this laboratory, 90–95% of the seeded lymphocytes are harvested by pipette mixing, but few of the monocytes are recovered, presumably because they adhere to the walls of the plastic microtitre plates.<sup>18</sup> The volume distribution of the control and stimulated cultures was measured with an electronic particle counter and a multi-channel analyser (Fn and C1000, Coulter Electronics, Harpenden, England). Because of cultural failures, no results are available for Con A stimulation on one sample from a cimetidine patient and from four samples from ranitidine patients. These profiles were analysed by computer to determine the percentage of the cells responding to stimulation and their averaged growth rate ( $a+bv$ ), where  $v$  is the volume of the cell and  $a$  and  $b$  are constants, named the basal and incremental growth rates respectively.<sup>18, 19</sup>

Table 1 Details of patients included in study

Treatment	Number	Males	Females	Mean age (yr)	Mean duration of ulcer history (yr)	Cigarette smokers
Cimetidine	18	13	5	43.2	8.9	14
Ranitidine	15	9	6	45.4	9.8	11

## PERIPHERAL BLOOD LYMPHOCYTE COUNTS

The white blood cell count was measured in the sequestrene blood sample with a Coulter (Model S) counter and the differential count was performed microscopically in Leishman-stained blood films.

## STATISTICAL METHODS

The findings of the three sampling times were subjected to a two-way analysis of variance using the Generalised Linear Interactive Modelling Package produced by the Royal Statistical Society.<sup>20</sup> The Tukey one-degree of freedom test<sup>21</sup> was used to assess the validity of the assumption of additivity. An additional series of non-parametric analyses using the Friedman rank test<sup>22</sup> was performed to assess the pattern of changes with time. This test was used as a back-up, as it is not sensitive to outliers. The Tukey median polish technique<sup>23</sup> was used to provide robust estimates of the change in percentage of responsive lymphocytes with time.

## Results

## PERIPHERAL BLOOD LYMPHOCYTES DURING TREATMENT WITH CIMETIDINE

The findings on numbers of lymphocytes circulating in the peripheral blood are summarised in Table 2. The mean white blood cell count fell during the period of study. For individual patients the mean reduction from the pretreatment level was  $1.1 \pm 0.4$  (SD)  $\times 10^9/l$  at two weeks and  $1.5 \pm 0.4 \times 10^9/l$  at four weeks: these differences were statistically significant ( $p < 0.01$ ). There were no consistent changes in the percentage of lymphocytes in the differential count: for individual patients the mean change was a fall of  $0.1 \pm 2.1\%$  at two weeks and a rise of  $1.5 \pm 2.3\%$  at four weeks and these differences were not statistically significant. The calculated absolute lymphocyte count fell during the period of study, but the mean fall from the pretreatment level for

Table 2 Summary of haematological findings in duodenal ulcer patients treated with cimetidine or ranitidine (data presented as mean  $\pm$  SD)

Drug	Duration of treatment (wk)	WBC ( $\times 10^9/l$ )	% Lymphocytes in differential count	Absolute lymphocyte count ( $\times 10^9/l$ )
Cimetidine	0	$8.1 \pm 2.1$	$32.5 \pm 7.4$	$2.5 \pm 0.7$
	2	$7.1 \pm 1.8$	$31.9 \pm 9.1$	$2.2 \pm 0.8$
	4	$6.5 \pm 1.6$	$33.8 \pm 8.0$	$2.2 \pm 0.7$
Ranitidine	0	$7.4 \pm 1.2$	$29.8 \pm 5.9$	$2.2 \pm 0.4$
	2	$7.1 \pm 1.6$	$30.4 \pm 6.7$	$2.1 \pm 0.6$
	4	$6.7 \pm 2.0$	$29.2 \pm 6.0$	$1.9 \pm 0.6$

individual patients was not statistically significant ( $0.42 \pm 0.49\%$  at two weeks and  $0.4 \pm 0.7\%$  at four weeks).

The findings on analysis of variance of results of lymphocyte stimulation tests are summarised in Table 3.

All patients responded in a comparable manner. None of the Tukey one-degree of freedom tests for non-additivity was significant. After treatment with cimetidine for two weeks there was a large and significant increase in the proportion of the peripheral blood lymphocytes that had entered G<sub>1</sub>-phase growth in response to stimulation with each of the three mitogens. After four weeks' treatment, the response to phytohaemagglutinin was still markedly and significantly increased, that to Con A was increased to a lesser but still significant ( $p < 0.05$ ) extent, but that to pokeweed mitogen was not significantly changed from the pretreatment level. The validity of these conclusions was confirmed, in pattern, by the Friedman rank tests and, in extent, by the Tukey median polish estimates. The changes in incremental growth rates were not statistically significant. The effects were unrelated to the sex of the patient or to the

Table 3 Results of analysis of variance of changes with time in responses of lymphocytes from patients with duodenal ulcer to mitogen stimulation during course of treatment

Changes in percentage of cells responding to mitogen stimulation							
Drug treatment	Mitogen	No. of patients studied	Variance ratio	Degrees of freedom	Probability	Mean increase in percentage of lymphocytes responding $\pm$ SD after treatment for	
						14 days	28 days
Cimetidine	PHA	16	4.83	2:24	<0.025	$12.0 \pm 4.6$	$12.3 \pm 4.6$
	Con A	17	5.88	2:26	<0.01	$13.8 \pm 4.0$	$7.6 \pm 4.4$
	PWM	18	5.93	2:27	<0.01	$15.6 \pm 4.6$	$3.7 \pm 4.7$ (N.S.)
Ranitidine	PHA	15	0.81	2:21	NS	$-7.1 \pm 5.7$	$-5.1 \pm 5.6$
	Con A	14	0.07	2:22	NS	$2.2 \pm 6.1$	$1.2 \pm 6.1$
	PWM	15	0.58	2:25	NS	$-3.8 \pm 4.9$	$-5.1 \pm 5.0$

achievement of ulcer healing during the period of study.

#### PERIPHERAL BLOOD LYMPHOCYTES DURING TREATMENT WITH RANITIDINE

The findings on numbers of lymphocytes circulating in the peripheral blood are summarised in Table 2. Although the mean white blood cell count fell during the period of study, the mean decrease from pretreatment levels was small ( $0.5 \pm 0.3 \times 10^9/l$  at two weeks and  $0.7 \pm 0.3 \times 10^9/l$  at four weeks) and did not achieve statistical significance. There were no consistent changes in the percentages of lymphocytes in the differential count: for individual patients, the mean rise at two weeks was  $0.8 \pm 1.8\%$ . The calculated absolute lymphocyte count fell during the period of study, but the changes for individual patients were not statistically significant (rise of  $0.15 \pm 0.72 \times 10^9/l$  at two weeks and fall of  $0.3 \pm 0.49 \times 10^9/l$  at four weeks).

The findings in the mitogen stimulation tests on lymphocytes for ranitidine-treated patients are summarised in Table 3. These are in complete contrast with the effects of cimetidine, as with ranitidine there were no significant differences either in percentage of cells responding or in their incremental growth rate with any of the three mitogens. The Friedman rank test results and the Tukey median polish estimates confirmed the two-way analysis of variance.

#### Discussion

We have shown an increased response to *in vitro* mitogen-stimulation in peripheral blood lymphocytes from duodenal ulcer patients during treatment with cimetidine. A greater proportion of cells enter G<sub>1</sub>-phase and the absence of change in incremental growth rate implies that the kinetics of recruitment into growth are unchanged.<sup>24</sup> It is not possible to deduce from our studies whether cimetidine, when administered orally, influences the lymphocyte directly (as would be implied by increased mitogen responses of normal lymphocytes seen after addition of the drug to the culture medium)<sup>7</sup> or whether it causes an *in vivo* redistribution between the pools of lymphocytes in the peripheral blood and the lymphoid organs or other solid tissues: the absence of any major changes in the absolute numbers of circulating lymphocytes argues against the latter mechanism.

The different response to the mitogens in the 14 and 28 day samples is of interest, as blood samples drawn at 14 days would be expected to contain significant quantities of active drug, while those

drawn at 28 days (14–16 hours after the last dose) would not be expected to contain detectable quantities of either drug.<sup>25 26</sup> Our findings, therefore, suggest that some, at least, of the immunological effects of cimetidine are rapidly reversed on stopping treatment.

Previous studies of human lymphocyte function in relationship to cimetidine have given contradictory results. While the *in vitro* studies of Gifford *et al*<sup>7</sup> produced findings broadly in accord with our own, Festen *et al*<sup>27</sup> were unable to show any effect of cimetidine treatment on lymphocyte function in nine patients with duodenal ulcer. Jorizzo *et al*<sup>28</sup> found that cimetidine treatment increased the *in vitro* lymphocyte response to candida, but not to mitogens, in patients with chronic mucocutaneous candidiasis. Cimetidine treatment appears to correct skin anergy in man<sup>29–31</sup> and there is anecdotal evidence that it may be immunostimulant in metastatic cancer<sup>32</sup> and in potentiating renal allograft rejection,<sup>33 34</sup> although the latter conclusion is disputed.<sup>35–37</sup>

In contrast with the marked and consistent effects of cimetidine we were unable to demonstrate any effect of treatment with ranitidine on lymphocyte function, despite using doses which produce profound inhibition of gastric secretion,<sup>38</sup> and produce ulcer healing. Ranitidine and cimetidine, used in doses which exert profound pharmacological effects at the classical H<sub>2</sub>-receptors,<sup>39</sup> nevertheless had very different effects on lymphocyte function, indicating that the effects of cimetidine on lymphocyte function might not be mediated by classical H<sub>2</sub>-receptors. Support for this hypothesis is provided by studies of Vickers *et al*<sup>40</sup> who found that nordimaprit (an analogue of dimaprit without intrinsic H<sub>2</sub>-agonist activity) had similar effects on histamine and dimaprit (a potent H<sub>2</sub>-agonist) on lymphocyte function. Eyre and Chand<sup>41</sup> have reviewed the evidence for two subclasses of H<sub>2</sub>-receptor in other systems.

Other situations have been reported in which cimetidine and ranitidine have different effects. Thus cimetidine, but not ranitidine, stimulates prolactin secretion on bolus injection,<sup>42</sup> increases serum testosterone levels,<sup>43</sup> and inhibits hepatic microsomal drug metabolism.<sup>44</sup> It remains to be seen whether any of these effects, which are not thought to be mediated at H<sub>2</sub>-receptors, are related to the effects of cimetidine on lymphocyte function. It appears that the imidazole nucleus of cimetidine provides the molecular basis for functional activity in these different systems and that the change from imidazole to the substituted furan nucleus in ranitidine abolishes these effects while retaining the antisecretory effects.

In conclusion, we have shown that treatment with cimetidine *in vivo* augments the response of lymphocytes from patients with duodenal ulcer to *in vitro* mitogenic stimulation, while treatment with ranitidine does not. These results suggest that the effect of cimetidine on lymphocytes is not mediated at classical histamine H<sub>2</sub>-receptors.

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