EFFECTS OF CIMETIDINE ON HAEMATOPOIESIS *in vitro*

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1   The possible modifications in haematopoiesis induced by cimetidine were studied in normal bone marrow cultures *in vitro*.

2   When cimetidine was added in the therapeutic range, there was no significant change in either granulocytic-macrophagic (CFU-GM) or erythroid (BFU-E) colony growth. However, when cimetidine was added to the culture at 2 to 25 times the therapeutic range, a small but significant inhibition of both types of colony growth was found.

3   We conclude that cimetidine in the therapeutic range does not induce inhibition of haematopoiesis *in vitro* but does in doses above the therapeutic range when inhibition is dose-dependent.

Introduction

The introduction of H_2-receptor antagonists represents a notable advance in the treatment of peptic ulcer disease. Of the early H_2-receptor antagonists, metiamide was considered the most effective, but it was abandoned after several cases of agranulocytosis were described (Isenberg 1976). This complication was related to the thiourea portion of metiamide. The substitution of thiourea by a cyanoguanidine group yields a new compound, cimetidine, which had no toxic effects on bone marrow, according to the earliest experimental and clinical studies. However in recent years, when cimetidine had gained widespread clinical use, cases of agranulocytosis (de Galocsy & van Ypersele de Strihov, 1979; Ufberg, Brooks, Rasanac & Kintzel, 1978), thrombocytopenia (Idwall, 1979), transitory pancytopenia (Gouffier, Schnurman, Durepaire & Vernant, 1978; James & Prout, 1978) and fatal pancytopenia (Chang & Morrison, 1979) were described in connection with the administration of cimetidine.

The purpose of this work was to assess the effect of cimetidine on human granulopoiesis and erythropoiesis, by means of *in vitro* bone marrow culture techniques.

Methods

In these experiments we used bone marrow samples from 14 patients who had bone marrow puncture performed for diagnostic reasons. The marrow samples were found to be normal.

Cimetidine was obtained from Smith, Kline & French, Philadelphia (Penn.) and diluted to the appropriate concentrations in phosphate-buffered saline.

Bone marrow culture studies

Bone marrow samples were collected in 2 ml of McCoy 5A medium containing 300 iu of preservative-free heparin in sterile tubes and after homogenization, were layered on Ficoll-Hypaque (density 1.077) and centrifuged at 800 g at 20°C for 10 min. The mononuclear layer was collected and washed 3 times with McCoy 5A medium.

Granulocytic-macrophagic colony (CFU-GM) assay

Human CFU-GM were assayed in 14 patients, as previously described by Robinson & Pike (1970); 10^6 normal unfractionated human leucocytes were plated on 0.5% agar-medium 5A and 1 ml aliquots in 35 mm Petri dishes and this layer was used to stimulate colony growth. Over this layer, 2×10^5 fractionated mononuclear bone marrow cells were plated in 0.3% agar-medium (McCoy 5A enriched with 20% foetal calf serum). After incubation at 37°C in a humidified atmosphere of 7.5% CO_2 in air for 10 days, granulocytic-macrophagic colonies of more than 40 cells were counted. Under these conditions, the mean number of colonies in our 14 normal samples was 134 for 2×10^5 plated cells (range 52–200).

Erythroid colonies: burst forming units (BFU-E)

Erythroid colonies were also cultured from 8 patients: 2×10^5 fractionated mononuclear cells were

plated in 0.3% agar-medium (McCoy 5A with 20% foetal calf serum) in aliquots of 1 ml in 35 mm Petri dishes, and 4 iu of erythropoietin (Step III, Conaugh, Toronto, Canada) was added to each plate. After incubation at 37°C in a fully-humidified atmosphere of 7.5% CO_2 in air for 17 days, bursts were scored. The mean number of bursts in these normal samples was 97 for 2×10^5 plated cells (range 25–195).

To plates for CFU-GM and BFU-E assays cimetidine was added at final concentrations of 2, 5, 10, 20, and 50 µg/ml before solidification, using cimetidine-free plates as controls. All scores were calculated using triplicate plates. The mean score of the three plates without cimetidine was considered as 100% CFU-GM and BFU-E formation, and therefore the scores of the plates with cimetidine were expressed as percentages of these. We assessed the variation between plates by calculating the percentage change between the lower and higher value of the three scored plates, thus obtaining a mean which was $4.0 \pm 2.5\%$ for CFU-GM and $6.8 \pm 2.5\%$ for BFU-E.

Statistical significance of the experiment was assessed by a paired Student's *t* test.

Results

The relationship between the cimetidine concentrations in culture and the percentage of growth inhibition of CFU-GM (14 normal bone marrow samples) and BFU-E (8 normal bone marrow samples) is shown in Table 1. A small but significant inhibition of BFU-E and CFU-GM was observed with concentra-

Table 1 Relationship between concentration of cimetidine in the culture and growth inhibition of BFU-E and CFU-GM

Cimetidine (µg/ml)	Colonies (BFU-E) (% of control)	Statistical significance (P)
2	100.80 ± 5.16	NS
5	95.25 ± 8.13	NS
10	83.68 ± 7.13	NS
20	82.25 ± 7.23	<0.05
50	76.83 ± 8.69	<0.05

Cimetidine (µg/ml)	Colonies (CFU-GM) (% of control)	Statistical significance
2	92.42 ± 4.16	NS
5	91.12 ± 3.88	<0.05
10	87.29 ± 3.87	<0.01
20	86.31 ± 3.97	<0.005
50	80.63 ± 4.42	<0.005

Values are given $\pm$ s.e.mean.

tions of cimetidine of 20 and 5 µg respectively. The percentage of growth inhibition increased progressively when higher doses of cimetidine were added to the culture. However, the percentage of growth inhibition was not very large even though significant at high concentrations of this drug.

There was an important variation between patients in terms of the inhibition capacity of cimetidine to inhibit CFU-GM and BFU-E growth. In 6 different samples (3 CFU-GM and 3 BFU-GM) the inhibition ranged from 35 to 60% at maximal concentration of cimetidine. In two of these CFU-GM assays, the maximal inhibition was observed at lower cimetidine concentrations and remained so when these were progressively increased.

Discussion

Our results show that cimetidine at levels similar to those used therapeutically fails to produce a significant inhibition of granulocytic macrophagic (CFU-GM) or erythroid colony (BFU-E) growth. When concentrations 2.5 to 25 times higher than the therapeutic levels were employed, discrete but significant inhibition of CFU-GM and BFU-E was obtained. These results confirm the inhibitory action of cimetidine on haemopoiesis in a dose-dependent fashion *in vitro*. This inhibition was first described by Posnett, Stein, Graber & Krantz (1979) and confirmed by Fitchen & Koeffler (1980), who used higher concentrations of cimetidine (10–20 times higher than the maximum used by us) and found high percentage or complete inhibition of CFU-GM *in vitro*, which was closely related to the cimetidine levels. These high levels of inhibition are only obtained with a toxic dose as in the case reported by Posnett *et al.* (1979) or if renal function is impaired (Ma, Brown, Masler & Silvus 1978). An individual susceptibility to cimetidine in human haemopoiesis cannot be ruled out here, since in the majority of the described cases with reversible or fatal cytopenias linked to this drug, the high levels produced by overdose or by impaired excretion could not be demonstrated. In fact, we have also observed this 'probable' individual susceptibility *in vitro*. Whether the serum levels of cimetidine are equivalent or not to those reached in the bone marrow microenvironment, remains unknown. Therefore, it is also unknown whether the persistence of the drug in the medullary microenvironment is dependent on its excretion.

The haematological toxicity of H_2-receptor antagonists has been attributed to the thiourea moiety and therefore it was expected that its suppression in the new generation of compounds (e.g. cimetidine), would abolish this side effect. However, the clinical

and *in vitro* results do not support this though the inhibitory action *in vitro* is notably higher in H_2-receptor antagonists with thiourea groups (e.g. metiamide) than in compounds without (e.g. cimetidine) (Fitchen & Koeffler, 1980).

Our results show that cimetidine not only acts on granulopoiesis, but also on erythropoiesis *in vitro* as previously found in clinical cases (Gouffier *et al.*, 1978; James & Prout, 1978; Chang & Morrison, 1979).

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