

CLINICAL RESEARCH

Mechanism of antihypertensive action of ketanserin in man

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**Abstract**  
A randomised double blind crossover study was carried out to determine whether ketanserin, a serotonin antagonist with an antihypertensive action in animals, has an  $\alpha_1$  adrenergic mediated antihypertensive effect in man. Steady state plasma ketanserin concentrations (mean 88 (SD 19)  $\mu\text{g/l}$ ) and cardiovascular responses measured in five healthy volunteers showed that therapeutic doses of ketanserin significantly antagonised the  $\alpha_1$  receptor mediated increase in arterial blood pressure after treatment with methoxamine.

Thus the antihypertensive action of ketanserin in man appears to originate from a blockade of peripheral vascular  $\alpha_1$  receptors.

Introduction

Ketanserin, which is under intensive clinical investigation for its antihypertensive effect,<sup>1</sup> potently displaces the ligand triethylamine spiperone from 5-hydroxytryptamine type 2 receptors<sup>2</sup> and is therefore characterised as a highly specific serotonin antagonist at 5-hydroxytryptamine type 2 receptors. As the effects of serotonin on the cardiovascular system are complex, with vasoconstriction in some and vasodilatation in other vascular beds,<sup>3</sup> and as there is only indirect evidence for the existence of 5-hydroxytryptamine type 2 receptors in vascular smooth muscle<sup>4</sup> the assumption that serotonin antagonism at peripheral vascular 5-hydroxytryptamine type 2 receptors is the mechanism of ketanserin's antihypertensive action is open to question and may represent only one of several possibilities. A physiological role of serotonin in centrally mediated regulation of blood pressure is being debated.<sup>5</sup> Although some adverse effects of ketanserin, such as dizziness, sedation, and

drowsiness, point to central actions of this drug, only one study of ketanserin given intracerebroventricularly has been reported and this did not show a reduction in blood pressure.<sup>6</sup> In addition to its 5-hydroxytryptamine type 2 antagonism ketanserin also has  $\alpha_1$  adrenergic activity in isolated blood vessels.<sup>7</sup> We therefore carried out a study to determine whether ketanserin has  $\alpha_1$  adrenergic effects in man.

Patients and methods

Five healthy volunteers (two men and three women, one of whom was a smoker) aged between 23 and 35 years participated in the trial after giving written informed consent. The study, which had been approved by the ethics committee of the Robert Bosch Hospital, was carried out in a double blind randomised crossover fashion. Dose response curves to methoxamine were established on two different occasions separated by at least one week, once during infusion of ketanserin and once during infusion of saline.

With the subjects in the supine position blood pressure (using a Riva-Rocci sphygmomanometer) and heart rate were measured at five minute intervals during a 30 minute equilibrium period. Subsequently, each subject received on one occasion a 10 ml bolus injection of saline (30 ml) followed by an intravenous infusion of saline (30 ml in 3 hours) and on the other occasion a bolus injection (three minutes) of 0.15 mg ketanserin in 10 ml saline followed by an intravenous infusion of 12 mg ketanserin in 30 ml saline (Janssen Pharmaceutica, Beerse, Belgium) in three hours. After 30 minutes' infusion of ketanserin or saline steady state concentrations were achieved and methoxamine hydrochloride (Vasoxyl, Barnagaia Welcome, Research Triangle Park, North Carolina, USA) was injected (15 second bolus) in a fixed dose sequence of 0.5, 1.0, 2.0, 4.0, and 8.0 mg dissolved in 2 ml saline at 30 minute intervals. After each injection of methoxamine blood pressure and heart rate were recorded at one minute intervals for the first five minutes after injection and at five minute intervals during the next 25 minutes until the next higher dose of methoxamine dose was injected. Before and at 30 minute intervals after the infusion had been started blood samples (10 ml) were drawn for analysis of plasma concentrations of ketanserin; blood was collected from the arm opposite the infusion site via an indwelling butterfly needle flushed with heparin in saline. After centrifugation plasma samples were frozen in -50°C aliquots and stored at -20°C. Ketanserin analyses were performed as described.<sup>8</sup>

Blood pressure and heart rate are expressed as means (SD) of the values obtained within the first three minutes after methoxamine injection. Mean arterial blood pressure was calculated as diastolic

Discussion

This study clearly shows that ketanserin has an  $\alpha_1$  anti-adrenergic action in man. Earlier studies of receptor binding with binding sites isolated from rat brain showed ketanserin to have an affinity to  $\alpha_1$  adrenergic receptors that was, however, only about a fifth that of 5-hydroxytryptamine type 2 receptors.<sup>2</sup> Thus interest focused on the 5-hydroxytryptamine type 2 receptor antagonism as the mechanism of the antihypertensive action of ketanserin. The effect of serotonin on vascular smooth muscle is difficult to predict; it depends on species, type of tissue, vascular beds, and basal vascular tone.<sup>3</sup> The effects of intravenously injected serotonin in man are by no means unequivocal, as shown by Hollander *et al.*<sup>5</sup> In 20 normotensive and 35 hypertensive subjects serotonin given in intravenous doses of 0.25-2.0 mg produced definite but variablepressor, depressor, or biphasic effects on arterial blood pressure. Thus it is difficult to establish the stable reduction of blood pressure observed in animals<sup>1</sup> and clinical studies<sup>9,10</sup> after administration of ketanserin solely on the basis of serotonin antagonism. Furthermore, recent studies in rats have provided evidence in favour of  $\alpha_1$  receptor blocking properties of ketanserin. Fozard<sup>11</sup> showed that in the pithed rat vasoconstriction in response to sympathetic stimulation was attenuated by ketanserin whereas the putative serotonin antagonist BW 501 C67 had no effect, although the doses of the latter were equivalent to those of ketanserin in producing a substantial blockade of serotonin receptors. Kalkman<sup>12</sup> showed that  $\alpha_1$  receptor mediated increases in blood pressure were similarly reduced by ketanserin and ketanserin, whereas neither substance changed the  $\alpha_2$  adrenoceptor mediated responses to B-HT 920. Consequently, the hypothesis that the antihypertensive action of ketanserin is based on a blockade of peripheral vascular 5-hydroxytryptamine type 2 receptors is not yet supported by reliable evidence.<sup>13</sup> From the results of our study we conclude that ketanserin shows  $\alpha_1$  adrenoceptor blocking properties in man at plasma concentrations that are normally obtained under therapeutic conditions. The antihypertensive action of ketanserin may thus be caused by its  $\alpha_1$  adrenoceptor antagonism.

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ONE HUNDRED YEARS AGO The throwing open of the competition for the designs of the new War Office and Admiralty has doubtless caused a flutter of excitement amongst architects of every grade. We note that of the judges of designs, one was the then Commissioner of Works, or, at least, will be an architect. It is too much to hope that there will also be placed on the committee a medical man of experience in hygiene and a sanitary engineer? In the conditions of competition, it is indeed laid down that "particular attention must be given to the general sanitary arrangements," which intending competitors will infallibly take to mean that the drains must not be forgotten, that the water-closets must be sufficient in number, and reasonably inodorous, and that the sewage must, at any rate have somewhere to go to. But "sanitary arrangements" in a pile of buildings such as is contemplated here, much more than that, and if there were sanitary experts on the committee of judges—and, for instance, Dr. Buchanan, the chief medical officer, and Sir Robert Rawlinson, the chief sanitary engineer of the Local Government Board, whose services would cost nothing—we might feel some confidence that the hideous blunders of the new Government offices in Parliament Street would not be repeated. "Sanitary arrangements" in reality involve the whole scheme of the buildings. They comprehend the disposition of the rooms as regards light and ventilation, the area and height of the rooms, the position and construction of the staircases, and other details that may affect very importantly the health and comfort of the inmates. Government officials, even if they be not the strictest workers of beings, have the right to demand reasonable comfort in their work. Moreover, it is a positive economy to the State to keep them in good health, since in their absence their duties must of necessity be provided for. In the new Government offices everything—light, ventilation, and other trifles—was sacrificed to an imposing facade; and as to the drainage, that was a matter not dreamt of in this connection. The present scheme, so unfortunately, seems to teach Government departments in the same bitter fashion that it does less-favoured mortals who have only their own money to play with, and it may therefore be useful to point to the moral of the previous Board of Work's undertaking of this kind, and to insist upon the necessity of the plans being critically examined by experts in sanitary science. (*British Medical Journal*, 1883; i:591.)

Correction

**Protective effect of vitamin E (DL- $\alpha$ -tocopherol) against intracerebral haemorrhage in presubhypertensive dogs**  
Two errors occurred in the first sentence of the abstract of this paper by Dr M. L. Chiswick *et al.* (July, p. 81). The sentence should have read: "Forty four males, of less than 37 weeks' gestation, were either randomly given 10 mg/kg vitamin E (DL- $\alpha$ -tocopherol) or placebo intramuscularly after birth (day 0) and on days 1, 2, and 3 or served as controls."

blood pressure (systolic-diastolic pressure). Differences in blood pressure and heart rate with the varying doses of methoxamine and with the infusions of saline for ketanserin were analysed for significance by paired Student's *t* test. A probability level of *p* = 0.05 was presumed to reflect significance.

Results

Ketanserin caused a shift to the right in the response of mean arterial blood pressure to increasing doses of the  $\alpha_1$  adrenoceptor agonist methoxamine (Fig. 1). The mean arterial blood pressure during the control periods of saline and ketanserin infusion were slightly, but not significantly, different (94.1 (SD 9.1) vs 90.0 (SD 4.1) mm Hg (Fig. 1). Whereas during saline infusion the mean arterial blood pressure increased, depending on the dose, from 94.1 (SD 9.1) mm Hg to 110.6 (SD 15.1) mm Hg with increasing doses of methoxamine up to 4 mg there was no increase in mean arterial blood pressure with this dose during ketanserin infusion. Only at the highest dose of 8 mg did the mean arterial blood pressure rise from 90.0 (SD 4.1) to 98.7 (SD 8.8) mm Hg during ketanserin infusion. This dose was not, however, given during saline infusion because of the adverse effects—such as palpitations, anxiety, and pronounced bradycardia ( $< 36$  beats/minute) observed in two volunteers. There were no significant differences in blood pressure in each subject with increasing doses of methoxamine during saline or ketanserin infusion. There were significant differences in the changes in mean arterial blood pressure at all dosages when the results for the saline and ketanserin infusions were compared, even though at the lower two doses of methoxamine the differences in pressure were too small to be detected. At the 4 mg dose of methoxamine the mean arterial blood pressure response during saline infusion was about 30 times higher than that during ketanserin infusion (table 1). During saline infusion systolic and diastolic blood pressures were unchanged after 0.5 and 1.0 mg doses of methoxamine but rose sharply and significantly after the 2 mg and 4 mg doses (table 1). By contrast,

no significant changes in blood pressure occurred after doses of 0.5-5 mg methoxamine during simultaneous ketanserin infusion. A significant increase in mean diastolic blood pressure from 77.9 (SD 4.5) to 84.7 (SD 9.3) mm Hg was observed during ketanserin infusion only with the 6 mg dose of methoxamine. Whereas mean systolic blood pressure increased significantly from 117.8 (SD 12.1) to 128.0 (SD 9.8) mm Hg after 2 mg methoxamine during saline infusion, over 6 mg methoxamine did not cause a significant increase in systolic blood pressure during ketanserin infusion.

Methoxamine produced significant decreases in heart rate at all the doses given in this study after saline as well as ketanserin infusion. The fall in heart rate was, however, less during ketanserin infusion, except with the 0.5 mg dose of methoxamine. The difference between the fall after ketanserin infusion and that after saline infusion reached significance (*p* < 0.02) only for the 4 mg dose of methoxamine (Fig. 2).

The three minute intravenous bolus injection of 0.15 mg ketanserin/kg followed by a continuous infusion at the rate of 4 mg/h resulted in steady state mean plasma ketanserin concentrations of 88.3 (SD 19.1)  $\mu\text{g/l}$ . In the non-transplant group there was also a significant reduction of herpes simplex virus infection in the oropharynx and oesophagus (two out of 19 patients as compared with 10 out of 20; *p* = 0.018). Herpes simplex virus was isolated in the acyclovir arm within a day after starting the trial in one patient, and the other failure was due to a virus with reduced sensitivity to acyclovir in a patient who had had several previous courses of the drug. The incidence of herpes infections after stopping treatment was low.

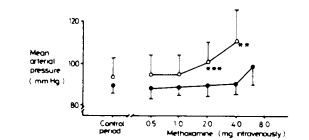


FIG. 1—Mean arterial pressure in response to increasing doses of methoxamine in five healthy volunteers given saline (○) and ketanserin (●) infusions. Bars represent SDs. \**p* < 0.01, \*\*\**p* < 0.001.

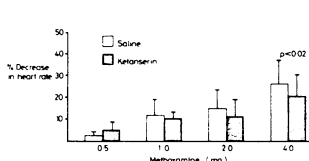


FIG. 2—Mean decrease in heart rate induced by methoxamine (expressed as % of control rate) during intravenous infusion with saline and ketanserin in the healthy volunteers. Bars represent SDs.

TABLE 1—Changes in mean arterial blood pressure (mm Hg) induced by doses of methoxamine during saline or ketanserin infusion

Case No.	Methoxamine dose (mg)				
	0.5	1.0	2.0	4.0	8.0
Saline infusion					
1	11.2	2.2	7.3	25.6	*
2	11.7	11.0	11.0	11.0	11.0
3	2.4	2.0	1.0	1.0	1.0
4	1.8	1.8	1.8	1.8	1.8
Mean (SD)	7.7 (4.1)	0.2 (4.1)	6.4 (1.1)	18.4 (9.9)	
Ketanserin infusion					
1	0.7	1.8	0.9	18.4	0.9
2	-4.9	1.8	2.7	0.7	7.3
3	1.8	1.8	1.8	1.8	1.8
4	0.7	0.7	0.7	0.7	0.7
5	1.0	1.0	1.0	1.0	1.0
Mean (SD)	1.5 (2.0)	1.7 (2.0)	1.4 (1.1)	15.4 (14.1)	4.0 (7.3)
Significance of difference between infusions (Student's <i>t</i> tested; <i>p</i> < 0.05)					
		<i>p</i> = 0.001	<i>p</i> = 0.003	<i>p</i> = 0.006	

\*Not given to volunteers during saline infusion because of side effects.

TABLE 2—Mean (SD) plasma concentrations of ketanserin and cardiovascular responses to intravenous injections of methoxamine during infusions of ketanserin and saline (*n* = 5)

Infusion	Control period	Doses of methoxamine (mg) (minutes after start of infusion)		
		0.5 (30)	2.0 (90)	6.0 (150)
Systolic blood pressure (mm Hg)				
Saline	117.8 (11.2)	118.3 (11.5)	119.2 (9.8)	128.0 (9.8)
Ketanserin	117.8 (16.2)	118.0 (10.0)	117.8 (10.0)	117.8 (11.4)
Diastolic blood pressure (mm Hg)				
Saline	77.9 (9.2)	81.1 (8.8)	82.4 (8.8)	84.7 (9.3)
Ketanserin	78.9 (6.5)	77.7 (6.5)	77.7 (6.5)	77.7 (6.5)
Heart rate (beats/min)				
Saline	64.0 (10.6)	62.8 (9.0)	56.4 (8.5)	54.0 (8.8)
Ketanserin	64.0 (10.6)	62.8 (9.0)	56.4 (8.5)	54.0 (8.8)
Plasma ketanserin concentration ( $\mu\text{g/l}$ )				
Ketanserin	90.0 (23.6)	86.2 (23.7)	87.1 (18.8)	88.0 (18.0)

Significance of difference compared with control observations (Student's paired *t* test). \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

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Acyclovir prophylaxis against herpes virus infections in severely immunocompromised patients: randomised double blind trial

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Abstract

Twenty patients undergoing allogeneic bone marrow transplantation and 39 patients receiving remission induction chemotherapy for acute leukaemia were entered into a double blind, placebo controlled stratified trial of acyclovir prophylaxis against herpes group virus infections. Within the transplant group intravenous acyclovir 5 mg/kg twice daily given throughout the period of granulocytopenia completely prevented oropharyngeal herpes simplex virus infection compared with a 50% incidence in the placebo arm (*p* = 0.003). The acyclovir group also had fewer days of fever during the trial and a shorter duration of leukopenia, possibly because of the prevention of herpes simplex virus infections. There was a high incidence of herpes infections after the trial in patients who received either acyclovir or placebo.

In the non-transplant group there was also a significant reduction of herpes simplex virus infection in the oropharynx and oesophagus (two out of 19 patients as compared with 10 out of 20; *p* = 0.018). Herpes simplex virus was isolated in the acyclovir arm within a day after starting the trial in one patient, and the other failure was due to a virus with reduced sensitivity to acyclovir in a patient who had had several previous courses of the drug. The incidence of herpes infections after stopping treatment was low.

The influence of acyclovir on excretion of Epstein-Barr virus in saliva in either group was inconclusive. One patient (transplant group) developed a cytomegalovirus infection while receiving acyclovir. Acyclovir provides effective prophylaxis against oropharyngeal and oesophageal herpes simplex virus infection in severely immunocompromised seropositive (>1/8) patients. In patients given bone marrow transplants this may have the additional benefit of reducing the time to recovery of an adequate blood count and the number of days of fever.

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Introduction

Patients with bone marrow failure either as a primary condition or after intensive chemotherapy or radiotherapy or both have a high incidence of herpes virus infections owing to reactivation of latent virus.<sup>1</sup> Usually these are superficial—for example, oropharyngeal and oesophageal for herpes simplex virus or cutaneous dermatome distribution for varicella zoster virus. These severely immunocompromised patients, however, frequently fail to contain the infection, which leads to dissemination such as pneumonia or encephalitis for herpes simplex virus and chickenpox with pneumonia for varicella zoster virus. In addition, patients undergoing bone marrow transplantation have a high incidence of cytomegalovirus infection (leading to hepatitis) and pneumonia (often fatal).

The incidence of herpes simplex virus infections in patients given bone marrow transplants was roughly 50%<sup>2</sup> in a previous group of 40 patients in our unit. A report from the Johns Hopkins unit quotes a figure of 45%<sup>3</sup> and from Seattle the combined incidence of herpes simplex virus and varicella zoster virus infections was 50%.<sup>4</sup> The Johns Hopkins Hospital report emphasised that acute lesions of herpes simplex occurred in only 14% of patients with a pretrial anti-herpes simplex virus serological titre of less than 1/8, whereas those with "positive" serological titres had an incidence of 73%. This supports the hypothesis that most herpes simplex virus infections after bone marrow transplantation are due to reactivation of latent virus. In a series of 525 transplant recipients in Seattle 12 died with herpes simplex pneumonia and eight with varicella zoster virus pneumonia.<sup>5</sup> The authors also noted that most herpes simplex virus infections occurred within the first month after transplantation but that infection with varicella zoster virus occurred with a median onset of 13 days after transplantation and continued to present for three to four years. Cytomegalovirus infection occurred mainly in the period three to six months after transplantation, and attempts at prophylaxis with adenine arabinoside (vidarabine) and treatment with human leucocyte interferon were unsuccessful.<sup>6</sup>

Acyclovir (9-(2-hydroxyethoxymethyl)guanine) is a guanine derivative with an acyclic side chain which is preferentially incorporated into cells infected with herpes viruses and transformed to its monophosphate by a specific viral thymidine kinase.<sup>7</sup> After further phosphorylation acyclovir triphosphate competes with deoxyguanosine triphosphate for incorporation into DNA and inhibits viral DNA polymerase to a greater extent than host DNA polymerase.<sup>8</sup> Acyclovir thus has a high "therapeutic index" and has proved active against herpes simplex virus and varicella zoster virus in vitro, against herpes simplex virus in laboratory animals, against herpes simplex virus and varicella zoster virus in man,<sup>9,10</sup> including the immunocompromised.<sup>11</sup> Epstein-Barr (EB) virus and cytomegalovirus do not code for a specific viral thymidine kinase, but nevertheless in vitro activity of acyclovir against EB virus with less than cytomegalovirus.<sup>12</sup> Therapeutic trials of acyclovir against cytomegalovirus, EB virus, and hepatitis B virus are at an early stage.<sup>13</sup>

The present study was designed to determine whether a twice daily infusion of acyclovir 5 mg/kg given throughout the period of granulocytopenia would prevent infection by the herpes viruses in immunocompromised patients.

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**Patients and methods**

All patients entering the allogeneic bone marrow transplantation programme at the Royal Free Hospital and having a herpes simplex virus titre of 1.8 or greater were entered into the trial over 14 months. The methods and techniques of transplantation were as described.<sup>12</sup> In brief, all 20 patients received high dose cyclophosphamide and total body irradiation using a linear accelerator before intravenous infusion of marrow from HLA-mixed leucocyte culture compatible, or minor mismatched, sibling donors. There were four patients with acute myeloid leukaemia and six with acute lymphoblastic leukaemia in the placebo arm, and six with acute myeloid leukaemia and four with acute lymphoblastic leukaemia in the acyclovir arm (table 1). All patients were nursed in reverse barrier isolation.

Similarly, all patients admitted for induction of remission of acute leukaemia were entered into the trial if they had a herpes simplex virus titre of 1.8 or greater and a creatinine clearance exceeding 30 ml/min. Two patients were ineligible because of impaired renal function. Forty patients were entered into the trial over 15 months, and one withdrew. There were 15 patients with acute myeloid leukaemia and five with acute lymphoblastic leukaemia in the placebo arm, and 12 with acute myeloid leukaemia and seven with acute lymphoblastic leukaemia in the acyclovir arm (see table 1).

After informed consent was obtained patients were allocated at random to receive acyclovir or placebo. Randomisation was stratified into two groups—allogeneic transplant recipients and acute leukaemic non-transplant recipients—by using a computer generated table of random numbers, which produced approximately equal numbers in each group of each stratification. The freeze dried sodium salt of acyclovir or equivalent placebo was dissolved in 100 ml isotonic saline (0.9% w/v) and infused (usually via an indwelling central venous Hickman catheter) over one hour. The dose of acyclovir was 5 mg/kg body weight every 12 hours given throughout the period of neutropenia (neutrophil count < 1.0 × 10<sup>9</sup>/l). All patients were examined daily under blind conditions by resident staff and any lesions recorded.

Urine specimens, throat swabs, and perianal swabs for viral surveillance were taken twice weekly from all patients throughout the trial. All possible viral lesions were cultured on 4 or alternate day basis. All of these specimens were taken into appropriate viral transport medium and immediately inoculated on to monolayer tissue cultures of human embryo lung cells in test tubes. The cultures were examined daily for 14–21 days. Viruses were identified by their pattern of cytopathic effect and by neutralisation and herpes specific fluorescent antibody staining was used to identify cytomegalovirus. Pre- and post-trial swabs were examined in parallel for a change in titre using a standard complement fixation test. Saliva specimens were taken weekly and cultured for EB virus using a modification of the method of Golden *et al.*<sup>13</sup>

An active herpes simplex virus infection was considered to have occurred when a patient developed mucosal ulceration or vesiculation from which the virus was isolated. When infection occurred the patient was treated with 5 mg/kg body weight hourly for five days. The diagnosis of cytomegalovirus infection was made when the typical clinical features (hepatitis, pneumonia, falling blood counts or pyrexia of unknown origin) coincided with a greater than fourfold increase in serological titre. Infection with varicella zoster virus was diagnosed by the typical clinical picture accompanied by a positive culture.

TABLE 1—Patient characteristics

	Bone marrow transplant group		Non-bone marrow transplant group	
	Placebo	Acyclovir	Placebo	Acyclovir
No. of patients	10	10	10	10
Age (years)	45.0	45.0	45.0	45.0
Mean weight (kg)	70.0	70.0	70.0	70.0
Mean leucocyte count (× 10 <sup>9</sup> /l)	0.0015	0.0015	0.0015	0.0015
Diagnosis				
Acute myeloid leukaemia	4	6	4	6
Acute lymphoblastic leukaemia	6	4	6	4
Male	6	6	6	6
Female	4	4	4	4
Single from isolation	0	1	0	1
Reverse isolation	10	10	10	10
2-5 bedded room	0	1	0	1
Mean herpes simplex virus titre at start (SE)	0.46 (0.11)	0.45 (0.11)	0.47 (0.10)	0.46 (0.11)
Mean No. of antibodies before trial (SE)	2.95 (0.11)	2.24 (0.10)	2.06 (0.11)	2.00 (0.11)

Viral sensitivity was tested by the methods of Collins *et al.*<sup>14</sup> The 50% inhibitory concentration (IC<sub>50</sub>) is defined as the concentration of acyclovir required to reduce the plaque count by 50% in comparison with untreated virus controls.

Blood samples were obtained one hour, eight hours, and 12 hours from the start of the infusion on day 1, and one hour and 12 hours from the start of the infusion on day 14. Plasma acyclovir concentrations were measured by the modification of the radioimmunoassay of Quinn *et al.*<sup>15</sup>

The two treatment groups were analysed separately. Patients in the placebo and acyclovir arms were compared for overall survival and within trial variables, in all cases a two tailed test was used.<sup>16</sup> Tests were used to compare the ratios of sex, diagnosis, and isolation stage. Age, weight, and lowest neutrophil and lymphocyte counts were compared using a test on log transformed data. The numbers of days on trial and at various stages of leukaemia and the number of antibiotics used were compared using tests on square root transformed data. This transformation was used to normalise the data. The proportion of patients with herpes simplex virus infection after entry into the trial was compared using Fisher's exact test and the results confirmed using a log rank test on time to virus shedding.<sup>17</sup> The groups were compared for biochemical and haematological values by analysis of variance.

**Results**

**BONE MARROW TRANSPLANT GROUP**

Ten patients received placebo and 10 acyclovir. The following variables were analysed: age, weight, lowest neutrophil and lymphocyte counts, herpes simplex virus titre at the start, and number of pretrial antibiotics (table 1).

The incidence of herpes simplex virus infection was significantly lower in the acyclovir group (zero versus 5 in the placebo arm; *p* = 0.033) (table 2). This protective effect was confirmed in the log rank test for time to herpes simplex virus infection (*p* = 0.01). In all five cases of infection the site was oropharyngeal but virus was also isolated from the urine and vagina in one patient, who had a generalised illness with oral and vaginal ulceration and fever. There was, however, no other definite evidence of disseminated infection. In addition, a sixth patient given placebo shed herpes simplex virus from the oropharynx with no oral ulceration. One patient taking acyclovir developed a fever, falling blood count and an increase in the anti-cytomegalovirus antibody titre. There were zero herpes group infections during the trial. These patients were followed up regularly for 10–24 months to document infections occurring after completion of the trial. There was a high incidence of herpes herpes simplex virus, varicella zoster virus, or cytomegalovirus infections in both arms after the trial (table 11). There was one virus related death due to cytomegalovirus pneumonitis and hepatitis in a 3 year old child after acyclovir prophylaxis.

The peak drug concentrations achieved were slightly higher on day 1 (mean value one hour after infusion 26.3 μmol/l, 59.2 μmol/100 ml) than on day 14 (mean one hour after infusion 11.9 μmol/l, 26.1 μmol/100 ml) but the difference was not significant. On day 1 the mean value eight hours after infusion was 5.2 μmol/l (11.7 μmol/100 ml).

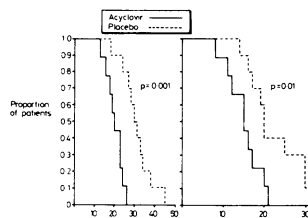
of days of fever (table IV) or in the numbers of days' use of antibiotics (mean of the square root of 2.06 (SE 0.11) days for placebo, 2.00 (SE 0.13) days for acyclovir).

The overall duration of granulocytopenia was similar in both groups (table V) but ten placebo patients with herpes simplex virus or cytomegalovirus infection were analysed separately they showed prolonged granulocytopenia (table VI). Drug concentrations were generally

TABLE VI—Association between herpes infections and prolonged granulocytopenia\*

Herpes infection	Bone marrow transplant group		Non-transplant group	
	Placebo	Acyclovir	Placebo	Acyclovir
Yes	5/5	1/1	6/6	2/2
No	5/5	9/9	4/4	10/10
Total	10	10	10	12

\*There was close correlation between acquisition of herpes virus infection and subsequent prolonged granulocytopenia in both groups (*p* < 0.05). Among patients in the transplant group this was more pronounced for granulocytopenia than for lymphopenia. Patients with cytomegalovirus infection and prolonged granulocytopenia in the non-transplant group differences were not significant.



slightly higher than in patients in the transplant group but not significantly so. As in the transplant group, the mean peak concentrations at the end of the infusion were higher on day 14 than at the start of the trial (28.3 μmol and 10.0 μmol/l (63.68 mg and 22.9 mg/100 ml) respectively), but this did not achieve statistical significance. On day 1 the mean concentration eight hours after the infusion was 4.4 μmol/l (9.9 μmol/100 ml) and 12 hours 1.2 μmol/l (2.7 mg/100 ml). On day 14 the mean value 12 hours after infusion was 14.4 μmol/l (32.4 mg/100 ml).

Analysis of biochemical and haematological values showed some minor differences significant at the 5% level at single doses within the trial. None of these were consistent throughout the trial. Two patients developed renal failure while receiving placebo and one while receiving acyclovir. Two of these cases were secondary to hyperuricaemia and one episode in a patient receiving placebo was probably related to chemotherapy for the malignancy. Two patients in the acyclovir arm during the trial, both of underlying disease and haemorrhage. One patient receiving placebo died of renal failure and hyperkalaemia with hyperuricaemia. Two patients receiving acyclovir and one receiving placebo were withdrawn from the trial after prolonged granulocytopenia due to refractory underlying disease.

Saliva specimens were tested for EB virus in 34 of the 30 patients in the non-transplant group and 18 in the placebo arm, 16 in the acyclovir arm. Five patients entering the study were excreting EB virus. Saliva samples became negative for the virus in four (two in the

placebo arm, two in the acyclovir arm). For one patient no follow up samples were available. One of the other patients had not been excreting EB virus on entry to the trial began to do so subsequently.

**Discussion**

This trial shows clearly that intravenous acyclovir 5 mg/kg twice daily given throughout the period of neutropenia is effective as prophylaxis against oropharyngeal and oesophageal herpes simplex virus infections in patients undergoing allogeneic bone marrow transplantation or remission induction chemotherapy for leukaemia. The reduction in incidence of these infections in patients in the transplant group from 50% to zero (table 1) was similar to that reported by Sarai *et al.*<sup>12</sup> Our study shows that the same may be achieved during remission induction treatment for acute leukaemia. This effect was achieved without any noticeable toxicity and, in particular, we were not able to confirm renal toxicity, which has been reported.<sup>12</sup> We may have avoided this problem by infusing the drug over one hour and ensuring good hydration, thus preventing the drug crystallising in the renal tubules.

Two of 19 patients in the acyclovir arm of the non-transplant group developed mucosal lesions of herpes simplex (table 11). One of these was documented shortly after prophylaxis began and the other was due to a virus of unknown origin. Infection with acyclovir in a patient who had had previous recurrent herpes simplex virus infections for which he had received two therapeutic courses of the drug. Concern has been expressed that widespread use of acyclovir to treat 'trivial' herpes simplex virus infections<sup>18</sup> may lead to the development and spread of resistant viruses. At present the frequency with which resistant viruses arise and their pathogenicity are imperfectly understood, although apparently they are unlikely to establish latency.<sup>19</sup> Also, they may be less pathogenic and might confer protection against reinfection with virulent strains.<sup>20</sup> This problem certainly deserves to be continually monitored with particular emphasis on whether infection with resistance strains occurs.

The use of acyclovir prophylaxis greatly reduced the morbidity associated with the treatment and of the underlying disease in our patients, although we did not encounter the type of disseminated infection with herpes simplex virus seen in the Seattle transplant series.<sup>4</sup> One patient in each stratification of our trial had a generalised illness, with herpes simplex virus being cultured from multiple sites and apparent response to acyclovir.<sup>21</sup> This cannot, however, be taken as conclusive evidence of disseminated herpes simplex virus infection. We found fewer overall days of fever in patients in the transplant group receiving acyclovir (table IV), and this was linked to fewer days of antibiotics for pyrexia of unknown origin (means of square roots 2.55 (SE 0.11) days for placebo, 2.24 (0.10) days for acyclovir; *p* = 0.06). The reduction in days of fever and antibiotics used for patients given acyclovir could also be due to protection against bactericidal infections, but we have no direct evidence for this. In the transplant group there were four cases of bacteraemia in the acyclovir arm and six in the placebo arm. In the non-transplant group there were eight and nine cases respectively. In larger groups of patients this effect could be studied more extensively. Theoretically, mucosal surfaces infected with virus predispose to bacterial infection because of enhanced adherence of pathogenic bacteria.<sup>22</sup> (P. Mackow *et al.* findings presented to 21st international conference on antimicrobial agents and chemotherapy, Chicago, 4-6 November 1981).

The other possible benefit of preventing infection with herpes simplex virus is the reduction in the period of leucopenia. Patients with prolonged granulocytopenia (< 1.0 × 10<sup>9</sup>/l for over 30 days) were analysed to see whether or not they had a herpes simplex virus infection during the trial (table V). Prolonged granulocytopenia was related to cytomegalovirus infection in the non-transplant placebo group (*p* = 0.019). The lack of significance in the transplant placebo group may have been due

and at 12 hours 1.0 μmol/l (2.25 mg/100 ml). On day 14 the mean value at 12 hours was 0.3 μmol/l (0.68 mg/100 ml). All viruses isolated were tested and shown to be fully sensitive to acyclovir (IC<sub>50</sub> 0.12–0.55 μmol/l, 1.24 mg/100 ml).

Analysis of the number of days of fever (table IV) showed a significant reduction in patients in the acyclovir arm at all granulocyte counts and at all but the highest lymphocyte counts. When the patient with cytomegalovirus infection and consequent prolonged granulocytopenia is excluded the duration of granulocytopenia was significantly shorter for patients in the acyclovir arm at counts of 0.10–0.40 and 0.51–1.0 × 10<sup>9</sup>/l. This reduction may be due to the acquisition of herpes simplex virus infection in patients in the transplant group (table VI, figure). Similarly, there was a reduction in the duration of lymphopenia at these counts.

Toxicity studies showed some minor differences at single dates within the trial. None of these were consistent throughout this period. Nausea and vomiting occurred in five patients treated with acyclovir and eight of those receiving placebo. There was no evidence of renal or hepatic toxicity and no patient was withdrawn from the trial. Two patients died during the trial, one from an acute gut (radiation induced) syndrome and another because of progressive underlying disease.

On entry to the trial five patients were excreting EB virus in their saliva; of these, four received acyclovir prophylaxis. One

patient (receiving acyclovir) had a positive result one week later, saliva samples subsequently becoming negative. All other patients stopped excreting EB virus, including the patient receiving placebo.

**NON-TRANSPLANT GROUP**

Twenty patients received placebo and 19 acyclovir. As in the transplant group, there were no significant differences in the patients' demographic details (table 1). Two patients given acyclovir acquired a herpes simplex virus infection compared with 10 of 20 given placebo (table 11). Of the two failures of prophylaxis with acyclovir, one occurred within 24 hours of starting the trial and began to resolve before 'therapeutic' acyclovir was needed. This infection was due to a fully sensitive virus (IC<sub>50</sub> 0.14–0.20 μmol/l; 0.32–0.45 mg/100 ml). The other infection occurred in a patient who had had recurrent herpes simplex virus infection and had received two previous therapeutic courses of acyclovir. Viral samples tested 10 days before the trial were sensitive (IC<sub>50</sub> 0.12–0.60 μmol/l; 0.27–1.35 mg/100 ml) but the organisms isolated during the trial showed reduced sensitivity (IC<sub>50</sub> 5.0–9.5 μmol/l; 11.25–21.38 mg/100 ml). This infection improved, however, with therapeutic acyclovir (5 mg/kg eight hourly). The protective effect of prophylactic acyclovir was confirmed by Fisher's exact test (*p* = 0.018) and the log rank test (*p* = 0.006).

	Bone marrow transplant group		Non-transplant group	
	Placebo	Acyclovir	Placebo	Acyclovir
No. of patients	10	10	19	20
No. of cases of herpes simplex virus infection	0	0	10	2
<i>p</i> value			0.033	0.018

TABLE II—Incidence of herpes simplex virus infections in the two groups of patients during trial

	Bone marrow transplant group		Non-transplant group	
	Placebo	Acyclovir	Placebo	Acyclovir
No. of patients	10	10	19	20
No. of cases of herpes simplex virus infection	0	0	10	2
<i>p</i> value			0.033	0.018

TABLE III—Viral infections after completion of trial

	Bone marrow transplant group		Non-transplant group	
	Placebo	Acyclovir	Placebo	Acyclovir
Herpes simplex	7	3	10	2
Herpes zoster	2	0	0	0
Cytomegalovirus	4*	2	0	0
Epstein-Barr	2	0	0	0
Total	14*	5	10	2

\*Includes two cases of pneumonia with hepatitis, one fatal. Multiple infections in five patients.

TABLE IV—Numbers of days of fever during trial. Results expressed as means of square roots (SE in parentheses)\*

	Bone marrow transplant group		Non-transplant group	
	Placebo	Acyclovir	Placebo	Acyclovir
Granulocytopenia (< 10 <sup>9</sup> /l)	1.58 (0.26)	2.33 (0.29)	0.047	1.21 (0.36)
Lymphopenia (< 10 <sup>9</sup> /l)	3.23 (0.24)	2.59 (0.30)	0.015	2.56 (0.28)
Lymphocytes (> 10 <sup>9</sup> /l)	1.90 (0.21)	2.90 (0.29)	0.07	2.03 (0.31)
Fever	1.92 (0.37)	1.68 (0.25)	0.1	2.00 (0.11)

\*Numbers of days of fever in various states of leucopenia compared using a test on square root transformed data. Transformation used to normalise data. Patients on transplant group showed reduction in duration of fever at all granulocyte counts and at intermediate lymphocyte counts.

TABLE V—Numbers of days of leucopenia during trial. Results expressed as means of square roots (SE in parentheses)\*

	Bone marrow transplant group		Non-transplant group	
	Placebo	Acyclovir	Placebo	Acyclovir
Granulocytopenia (< 10 <sup>9</sup> /l)	5.51 (0.21)	4.47 (0.16)	0.001	4.56 (0.39)
Lymphopenia (< 10 <sup>9</sup> /l)	4.06 (0.22)	3.68 (0.19)	0.017	4.46 (0.41)
Lymphocytes (> 10 <sup>9</sup> /l)	5.19 (0.26)	6.05 (0.23)	0.027	4.46 (0.41)
Fever	5.57 (0.25)	4.45 (0.14)	0.025	4.40 (0.42)
Antibiotics	5.19 (0.26)	4.45 (0.16)	0.025	4.40 (0.42)
Total	3.48 (0.36)	2.50 (0.22)	0.01	5.50 (0.22)

\*Results exclude one patient given acyclovir prophylaxis who had prolonged leucopenia associated with cytomegalovirus infection. Numbers of leucopenic days analysed using positive tests on square root transformed data. There was shorter duration of granulocytopenia and lymphopenia for patients in transplant group at all except lymphocyte counts.

to the number of patients concerned. The cumulative frequency curves of granulocytopenic days in the transplant group (figure), which was confirmed by *t* test on the mean number of days with granulocytopenia (< 1.0 × 10<sup>9</sup>/l; *p* = 0.001) and < 0.5 × 10<sup>9</sup>/l (*p* = 0.01). The reason for this virus' precludes any possibility of successful prophylaxis when this is confined to the granulocytopenic period after transplantation. The lack of sensitivity of cytomegalovirus to acyclovir in vitro makes it unlikely that prolonged prophylaxis would be successful.<sup>23</sup> Similarly, infection with varicella zoster virus may occur up to several years after transplantation,<sup>24</sup> and thus prophylaxis with intravenous acyclovir would not be feasible.

Although of considerable theoretical interest, the data on excretion of EB virus must be considered inconclusive. There appears to be no correlation between excretion of the virus and the type of treatment given. Studies on the therapeutic effect of acyclovir in EB virus infections would perhaps throw more light on this problem.

Acyclovir does not prevent latency of herpes simplex virus, as evidenced by the high rate of recurrent infection in this series, confirming the findings of Sarai *et al.*<sup>12</sup> In both series, however, there was evidence that the recurrences are often clinically mild. We conclude that prevention of herpes simplex virus infection during the period of neutropenia is achievable with acyclovir and of sufficient clinical benefit to be warranted.

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ONE HUNDRED YEARS AGO At a time when so many real and imaginary dangers are discussed by sanitarians, it is surprising to say our contemporary *Archives* that no one has called attention to an arrangement that is common in some, if not in all, the public baths of London, as well as in provincial towns, and which appears to be