metacarpals throughout the two years of observation. The oestrogen-treated group lost none, and loss in the calcium-treated group was intermediate.

The main effect of oestrogen on the metacarpals was inhibition of endosteal bone resorption, possibly with stimulation of subperiosteal bone apposition. The slight increase in total bone width transformed a very small, non-significant decline in cortical width into a small, non-significant increase in cortical area. Cortical area is the better estimator of bone quantity,¹⁰ and when the results are expressed in this way full account is taken of the effects of the treatment at both cortical surfaces. The positive mean $\Delta\overline{CA}/t$ value in the oestrogen-treated group was consistent with the densitometric observations of slight (although again non-significant) increases in the mineral contents of the ulna and radius.

Our results confirm the outcome of the prospective trial of oestrogen (mestranol) treatment in oophorectomised women reported by Aitken *et al.*⁶ These authors, however, suggested that oestrogen treatment is effective only when instituted within three years of the menopause, since in a group six years from the menopause oestrogen did not significantly change the rate of bone loss. An explanation for this may be that in untreated women there is a phase of rapid bone loss immediately after the menopause,¹¹ in which a significant effect of oestrogen treatment would be easier to see. Although the mean time since the menopause in our patients was about six years, all the groups contained a high proportion of women within three years of the menopause. This explains why our control group lost bone at a rate considerably higher than controls in previous series.⁹ Thus in our trial, as in that of Aitken *et al.*, the detection of a response was facilitated by studying groups of women relatively close to the menopause, but we do not conclude that oestrogen treatment at a later stage would be ineffective.

The data on the calcium-treated group are slightly less consistent. A significant effect of calcium treatment was shown on the ulna, and the mean rate of loss of bone mineral from the radius was also reduced, although not significantly. The morpho-

metric data on the metacarpals, when expressed as changes in cortical width, disclose only a small, non-significant effect of calcium. Because the effect of calcium treatment appeared to be confined to the periosteal surface, however, the effect on cortical area was more pronounced. The mean rate of decrease in mean cortical area in the calcium-treated group was less than half that in the control group, with the difference approaching significance; it was not significantly different from the mean rate in the oestrogen-treated group, however.

We thank Professor M S F McLachlan, head of the University Department of Radiodiagnosis, Leeds, and H B Bentley, principal of the School of Radiography, Leeds General Infirmary, for help with the radiographic aspects of the trial. We are also indebted to P A Kirby and R M Milner for technical help.

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(Accepted 29 July 1977)

Immunosuppressive properties of sera and urine dialysates from kidney-graft recipients treated with azathioprine, prednisolone, and niridazole

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British Medical Journal, 1977, **2**, 792-795

Summary

Niridazole, an antischistosomal agent, was given to renal transplant recipients in addition to azathioprine and prednisolone, as there is experimental evidence that this combination of drugs is highly immunosuppressive. Sera obtained from kidney-graft recipients during the first two weeks after transplantation were examined for their ability to inhibit the one-way mixed lymphocyte reaction (MLR). Sera from seven patients receiving azathioprine, prednisolone, and niridazole (triple-drug treatment), five patients receiving azathioprine and prednisolone, and two other patients treated with niridazole alone for schistosomiasis produced MLR inhibition by comparison with pretreatment (control) sera.

A mean of 78% inhibition was observed with sera taken after one day's treatment with the three-drug combination, whereas this level of in-vitro immunosuppression

occurred only after eight days of treatment with azathioprine and prednisolone. Niridazole alone produced an effect similar to azathioprine and prednisolone. Concentrated dialysate of urine from a patient receiving triple-drug treatment not only inhibited the MLR but also significantly prolonged the survival of heterotopic heart allografts in rats, whereas dialysate from the same patient after niridazole had been stopped gave less MLR inhibition and failed to prolong heart allograft survival.

Since niridazole thus increased the in-vitro and in-vivo immunosuppressive action of azathioprine and prednisolone, we suggest that this triple-drug combination might be useful for preventing early acute kidney graft rejection.

Introduction

The results of kidney transplantation remain disappointing,¹ despite better understanding of the mechanisms of graft

rejection, improvements in histocompatibility matching, and the accumulation of considerable surgical experience. Even when used in combination, immunosuppressive drugs in current use are not sufficiently powerful to prevent the loss of many grafts by uncontrolled rejection. Recent reports have suggested that an antischistosomal agent, niridazole (1-(5-Nitrothiazol-2-yl)imidazolidin-2-one), is immunosuppressive in mice²⁻³ and man,⁴ and inhibits cellular²⁻⁴ rather than humoral⁵ immune responses. Niridazole has been successfully used to prolong skin allograft survival in mice³ and heterotopic heart allografts in rats,⁶ and the latter study showed that synergism may exist between azathioprine, prednisolone, and niridazole. We therefore began a limited study of the immunosuppressive effects of these three drugs in various combinations in kidney-graft recipients and patients with schistosomiasis.

Patients and methods

Two patients with schistosomiasis were treated with 1500 mg niridazole daily for seven days. Five kidney-graft recipients received 150-100 mg azathioprine and 150-40 mg prednisolone per day in tapering doses over the first two weeks after transplantation. Seven kidney-graft recipients received similar doses of azathioprine and prednisolone, plus 1500 mg niridazole on alternate days starting on the day of transplantation (triple-drug regimen).

Sera obtained from all patients on the day before starting immunosuppressive treatment and samples obtained daily over the next two weeks when possible, were complement-inactivated at 56°C for 20 minutes, sterilised by filtration through 220-nm Millipore filters, and stored at -20°C in small aliquots. Sera were routinely added at a concentration of 10% (v/v) to human one-way mixed lymphocyte reactions (MLR), using 10⁵ responder (R) and 10⁵ mitomycin-treated stimulator (S_m) Ficoll-Hypaque separated mononuclear cells from healthy volunteers. In a few cases a range of concentrations was tested to obtain dose-response curves. MLRs were incubated for five days at 37°C in Sterilin microculture plates and were pulsed with 1 µCi ³H-thymidine (specific activity 2 Ci/mmol) for the last 18 hours. ³H-thymidine incorporated into dividing lymphocytes was recovered on to glass-fibre filter discs using the SAM II semiautomated harvester, and these were transferred to glass phials containing toluene-based liquid scintillation cocktail. Samples were counted for 10 minutes (counts per minute; cpm) on the Intertechnique SL-30 β-counter. Percentage MLR inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{(\text{cpm } R + S_m - \text{cpm } R + R_m) \text{ in control (pre-treatment) serum} - (\text{cpm } R + S_m - \text{cpm } R + R_m) \text{ in test serum}}{(\text{cpm } R + S_m - \text{cpm } R + R_m) \text{ in control serum}} \times 100$$

Twenty-four-hour urine samples were collected from one further kidney graft recipient while he was receiving triple-drug treatment, and again six days after niridazole had been withdrawn. The dosage of azathioprine (100 mg/day) and prednisolone (30 mg/day) was not altered. Urines were extensively dialysed against distilled water, rotary evaporated to dryness, and reconstituted to 100 times the original concentration of urine. Dialysates were either diluted further for addition to MLRs, or injected daily into AS inbred rats bearing Wistar heterotopic heart allografts⁶ in doses of 0.3 ml intraperitoneally. The heart grafts were palpated daily, and when the heart beat became difficult to feel cardiac activity was monitored using an electrocardiograph. Rejection was taken as the time when cardiac activity ceased.

Results

Representative dose-response curves for sera from the 14 patients

obtained on the sixth day of treatment are shown in fig 1. All cultures contained a total of 10% serum, which was composed of varying proportions of control:test serum to give the final concentrations of test serum shown. All three sera gave approximately linear dose-related MLR inhibition, with triple-drug serum producing substantially greater MLR inhibition at day six than either of the other two sera at all concentrations.

FIG 1—Dose-response curves for percentage inhibition of one-way mixed lymphocyte reactions (MLR) by sera from 14 patients treated with niridazole (Nir), azathioprine and prednisolone (Az + Pred), or niridazole, azathioprine, and prednisolone (Nir + Az + Pred). Each point is mean of four MLR combinations (AB_m, BA_m, CD_m, DC_m). Ranges shown as vertical bars.

Depression of MLR stimulation by sera obtained one to 13 days after starting immunosuppressive treatment compared with pretreatment sera occurred in all patients treated with any of the three regimens (fig 2). In patients with schistosomiasis who received only niridazole MLR inhibitory serum factors accumulated gradually during treatment to give a maximum of 70% MLR inhibition on day seven. Sera from patients on azathioprine and prednisolone showed a similar pattern of reactivity, reaching approximately 60% MLR inhibition on day seven of treatment and almost 100% inhibition on day 13. When sera from patients receiving triple-drug treatment were tested in this system six out of seven patients had almost 100% inhibition on day one of treatment and maintained high concentrations of MLR inhibitory factors throughout the period of testing. The seventh patient failed to produce high concentrations of inhibitory factors until day five of treatment, and thereafter had rather lower percentage MLR inhibition values than the other patients in this group.

Figure 3 shows the effect on the one-way MLR of urine dialysates from the same patient, which had been collected from him during triple-drug treatment and when niridazole had been withdrawn. In both cases dose-related MLR inhibition was obtained. Triple-drug dialysate was the more potent inhibitor *in vitro*. Azathioprine and prednisolone urine dialysate failed to prolong the survival of heterotopic heart allografts in rats, whereas significant ($P < 0.01$, Kruskal-Wallis analysis of variance) prolongation was obtained using triple-drug urine dialysate (table).

Discussion

Our results suggest that sera from kidney transplant recipients treated with azathioprine and prednisolone or the triple-drug regimen, or from patients with schistosomiasis treated with niridazole alone can suppress the MLR. Since this reaction has been proposed as an *in-vitro* model of the initial stages of the

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allograft response,⁷ the ability of patients' sera to suppress the MLR might indicate the presence of circulating factors potentially capable of preventing sensitisation to graft isoantigens. MLR inhibitory factors were also present in urine from a patient treated with the triple-drug regimen, and when a dialysate of this urine was given to rats at an arbitrary dose, slight but

Survival of heterotopic heart allografts in groups of AS rats

Group:	Untreated	Azathioprine and prednisolone	Niridazole, azathioprine, and prednisolone
Survival (days):	6 7 7 8 8 8 9	7 8 8 8 9 10 10	9 9 10 10 10 15 16
Mean (± SD)	7.6 ± 1.0	8.3 ± 0.9	11.1 ± 2.9
P value*		NS	<0.01

*Kruskal-Wallis analysis of variance.
NS = Not significant.
Rats were either untreated or given 0.3 ml urine dialysate (reconstituted to 100-times original concentration) intraperitoneally daily from time of operation. Dialysate was collected from a kidney-graft recipient while he was receiving niridazole, azathioprine, and prednisolone and six days after niridazole was withdrawn.

FIG 3—Dose-response curves for percentage inhibition of one-way mixed lymphocyte reactions (MLR) by urine dialysates from a patient treated with niridazole, azathioprine, and prednisolone (Nir + Az + Pred) and for the same patient after niridazole had been withdrawn (Az + Pred). Concentrations are by comparison with neat urine (urine dialysate reconstituted to original volume of urine equal to 100%). Each point is mean of four MLR combinations. Ranges shown as vertical bars.

FIG 2—Inhibition of one-way mixed lymphocyte reactions (MLR) by sera obtained over first two weeks of treatment from two patients with schistosomiasis receiving niridazole, five kidney-graft recipients treated with azathioprine and prednisolone, and seven treated with niridazole, azathioprine, and prednisolone. Graphs on left show MLR stimulation results (counts per minute (cpm) responder cells (R) + mitomycin-treated stimulator cells (S_m)) for individual patients. Mean cpm for all unstimulated cultures was 551 ± SD 289. Each point is mean of two MLR combinations. Graphs on right show mean % MLR inhibition in each patient group.

significant prolongation of heterotopic heart allograft survival across a strong histocompatibility barrier ($\text{AgB}^2 \rightarrow \text{AgB}^1$) resulted. Urine dialysate from the same patient six days after niridazole was withdrawn failed to prolong heart allograft survival.

To avoid graft rejection high concentrations of circulating immunosuppressive drugs or drug metabolites should preferably be established in the recipient's blood by the time of operation since the process of allograft rejection begins when the graft circulation is restored. Our results suggest that the addition of niridazole to the more usual immunosuppressive regimen of azathioprine and prednisolone ensures the presence of high concentrations of such factors within 24 hours of starting treatment. A much slower accumulation of immunosuppressive factors occurs when niridazole is omitted.

The immunosuppressive potential of niridazole has received attention only recently. We⁸ and others⁹ have shown that the parent compound has no immunosuppressive activity in vitro, whereas metabolites present in serum and urine from animals treated with niridazole are potent inhibitors of lymphokine production by antigen-sensitised lymphocytes⁹ and of the human MLR,⁸ and appear to act early in the antigen-recognition phase of the in-vitro reaction.⁸⁻¹⁰ While niridazole is rapidly broken down in vivo, its metabolites have a half-life of about 40 hours,¹¹ which we believe justified our use of alternate-day treatment in kidney-graft recipients. Toxic side effects prevent

the prolonged use of niridazole,¹² and we wished to identify the active metabolite(s) in the hope that these would be free from harmful side effects and might be given to transplant recipients for prolonged periods.

This study was supported by grants from the Medical Research Council and the Kidney Research Foundation for Wales.

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(Accepted 5 August 1977)

Cimetidine in patients with gastric ulcer: a multicentre controlled trial

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British Medical Journal, 1977, **2**, 795-799

Summary

Forty-five adult outpatients with endoscopically confirmed gastric ulceration completed a double-blind trial of either cimetidine (1 g/day) or placebo. After six weeks 18 of the 23 patients receiving cimetidine showed complete ulcer healing compared with only six of the 22 patients receiving placebo. The cimetidine group also had fewer days with pain than the placebo group but the difference was not statistically significant.

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Cimetidine therefore seems to promote healing of gastric ulcers without severe side effects, although its effect on pain is less pronounced than in patients with duodenal ulcers.

Introduction

The therapeutic effect of the histamine H_2 receptor antagonist cimetidine in duodenal ulcer is now well established,¹⁻³ whereas the drug's effect on gastric ulcer has received less attention. One early trial showed that cimetidine promoted ulcer healing,⁴ and two preliminary studies supported this finding, although the results were not conclusive.⁵⁻⁶ We present here the results of a double-blind study set up to assess the therapeutic efficacy of oral cimetidine, 1 g/day, compared with placebo in the short-term treatment of gastric ulcer.

Patients and methods

Fifty-one consecutive outpatients (18 women and 33 men) who fulfilled the following entry criteria were admitted to the trial. Firstly, a gastric ulcer had been seen at gastroscopy not more than seven days before start of treatment. All craters located above the pylorus were regarded as gastric ulcers, whereas craters in the pyloric canal were excluded. Secondly, no patient had had gastric surgery. Thirdly, there was no clinical or biochemical evidence of renal, hepatic, or cardiac disease (the laboratory investigations before entry included haemoglobin measurement; white and red blood counts; differential count; platelet count; estimation of serum aspartate aminotransferase, alkaline phosphatase, bilirubin, and creatinine