Biochemical indices of response to hydroxychloroquine and sodium aurothiomalate in rheumatoid arthritis

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SUMMARY   Biochemical and clinical changes have been monitored in 30 patients with rheumatoid arthritis treated with either hydroxychloroquine or sodium aurothiomalate over a period of 6 months. Acute-phase reactants improved in both treatment groups, while serum sulphydryl and serum histidine improved only in the gold-treated patients. Correlation matrices were constructed from mean clinical and biochemical data at successive clinic visits. Correlations obtained with gold were more frequent and of a higher level of significance than those obtained with hydroxychloroquine at the doses we studied. This lends support to the use of correlation matrices as a screening test for potential long-term antirheumatoid activity of drugs in man.

In an attempt to define biochemical and clinical changes that occur when patients with rheumatoid arthritis are exposed for the first time to 'specific antirheumatoid' therapy, and to see if these changes differ between drugs we have performed serial biochemical and clinical assessments over the first 6 months of treatment with a series of drugs for which antirheumatoid action has been claimed. Our results for groups of 15 patients treated with D-penicillamine[1][2] and alclofenac[2] have been described. We have now added 2 further groups of patients treated with hydroxychloroquine sulphate (HCQ) and sodium aurothiomalate (gold) in order to compare possible differences in biochemical response and to consider whether any particular changes are indicative of specific antirheumatoid effect. We have previously suggested that comparison of correlation matrices, constructed between mean data for biochemical and clinical variables, has relevance as a screening test for antirheumatoid activity.[2] We have therefore compared similar matrices for HCQ and gold.

Patients and methods

PATIENTS

Fifteen patients (13 female, mean age 51·8, range

39 to 66 years; 2 male, aged 31 and 49 years) were allocated to HCQ therapy, and a further 15 patients (11 female, mean age 52·7, range 36 to 67 years; 4 male, mean age 54·5, range 45 to 64 years) were allocated to gold therapy. All patients had classical or definite RA (American Rheumatism Association criteria) and at least moderate disease activity previously defined[1][2] by the presence of at least 3 of the following 5 criteria: (*a*) tenderness of more than 6 joints; (*b*) swelling of more than 3 joints; (*c*) morning stiffness longer than 45 minutes; (*d*) articular index more than 20; (*e*) ESR more than 28 mm. h^{-1}. None of the patients had received specific antirheumatoid drug therapy (e.g., gold, penicillamine, HCQ) before the present study. Patient exclusions were also as previously described.[1][2]

DRUG DOSAGE

Over a 24-week period HCQ was given in a dose of 200 mg b.d. and gold in a dose of 50 mg.week^{-1} intramuscularly until 1g had been given; then 50 mg. month^{-1} irrespective of clinical response.

Both groups received enteric coated aspirin in a dose of 3·6g.day^{-1} in the 2 weeks immediately preceding the study to establish 'baseline' conditions for clinical and systemic variables. During the 24 weeks of the study patients took supplementary enteric coated aspirin as required. However, 1 patient in each group took dextropropoxyphene and paracetamol tablets (Distalgesic) in place of aspirin
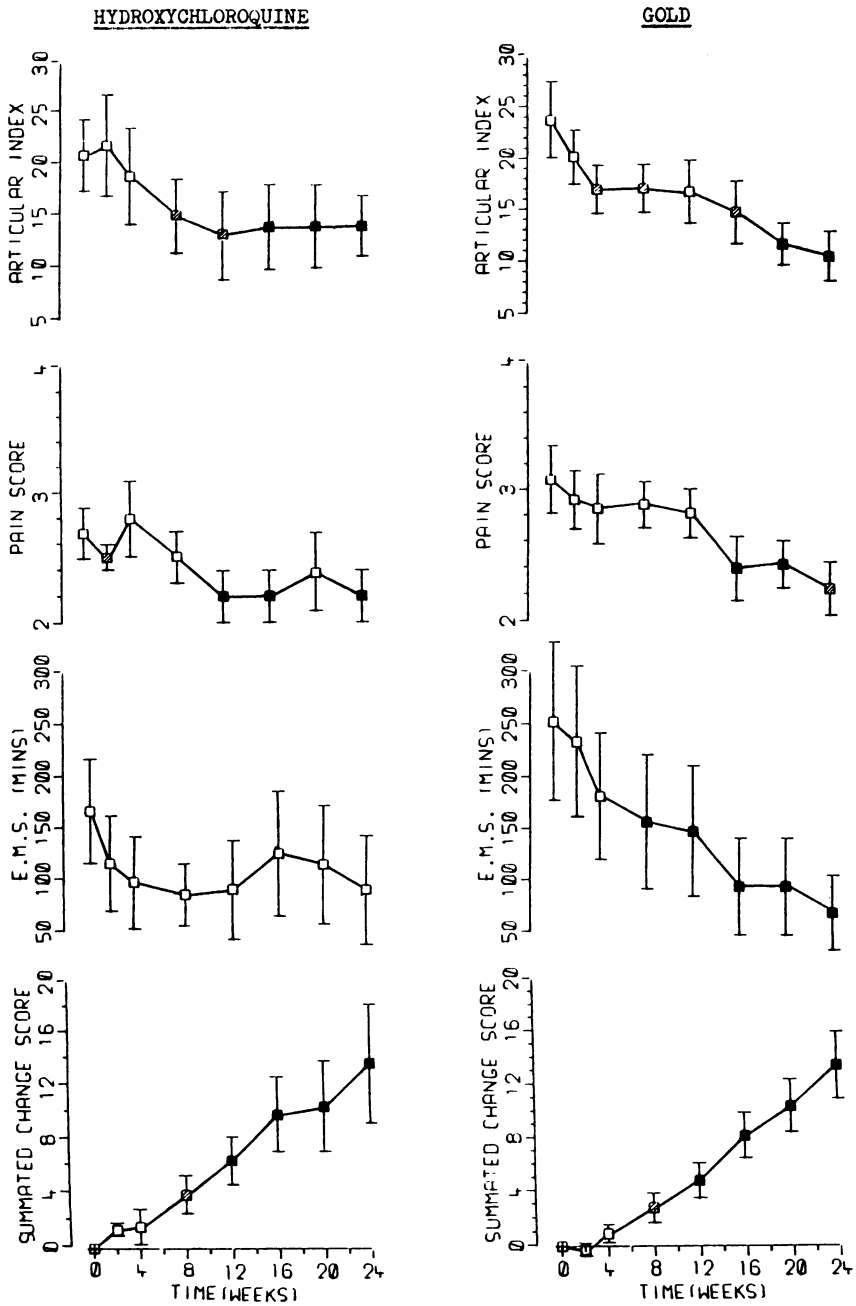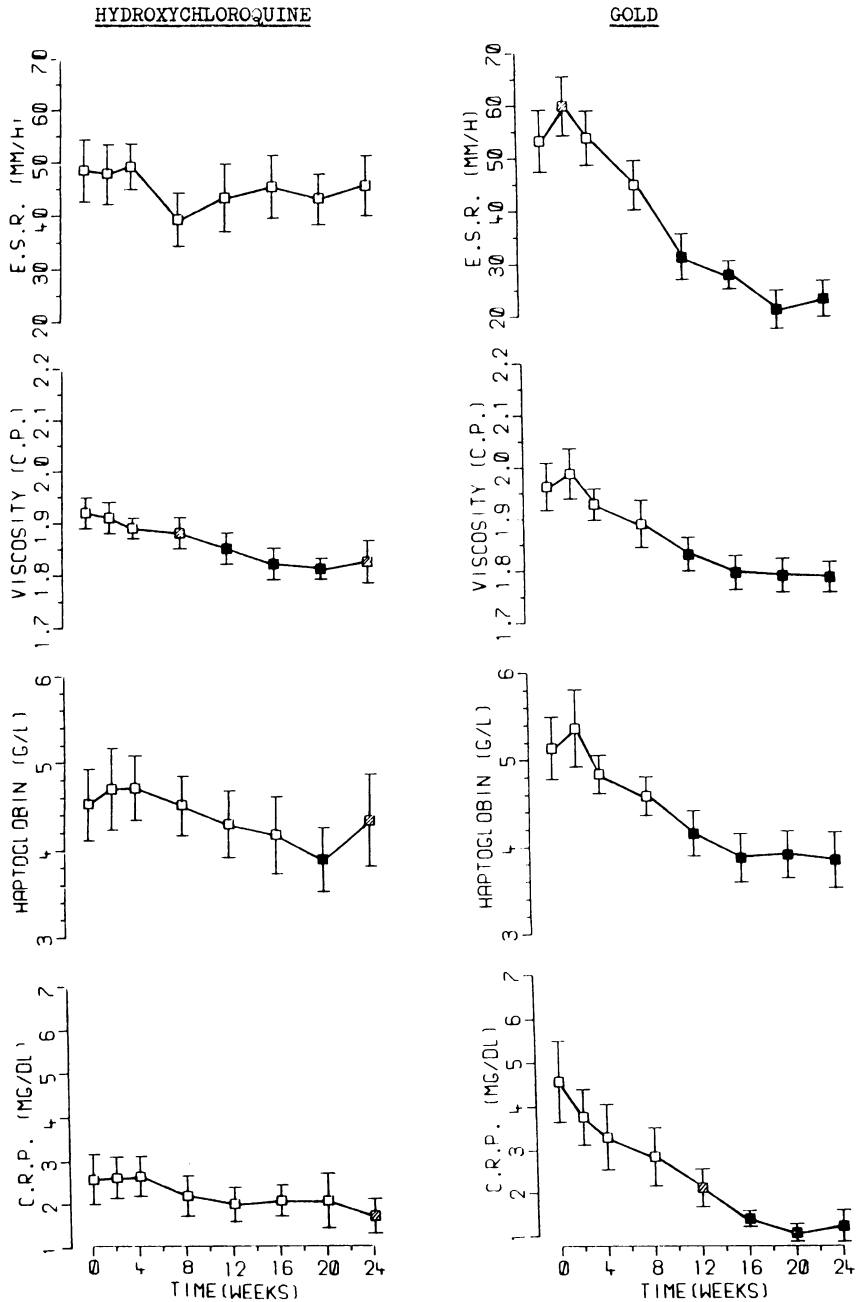
Fig. 1 *Clinical data (mean ± SE) for rheumatoid patients treated with hydroxychloroquine (left) and sodium aurothiomalate (right). Changes in individual parameters reaching statistical significance (Wilcoxon rank sum test) when compared with data at week 0 are indicated by hatched (p<0·05) and closed (p<0·01) data points.*
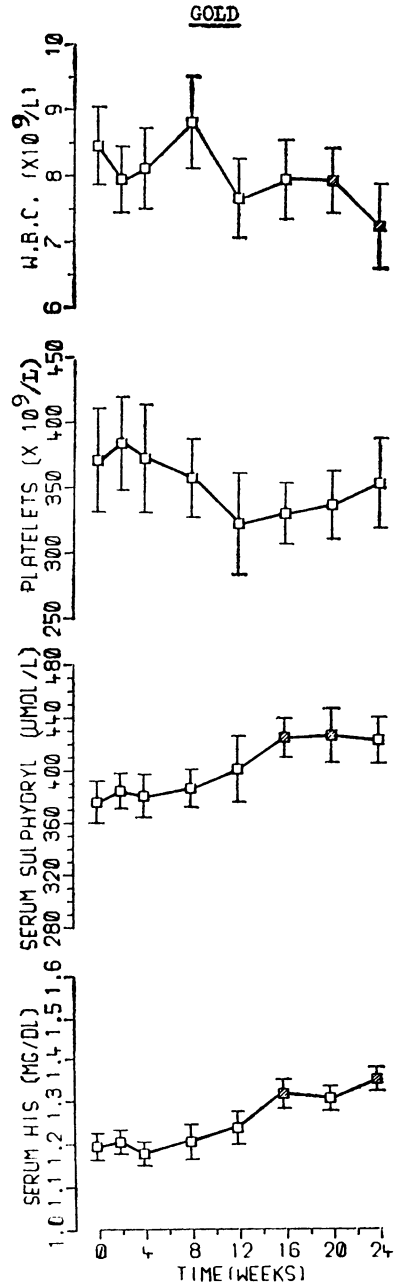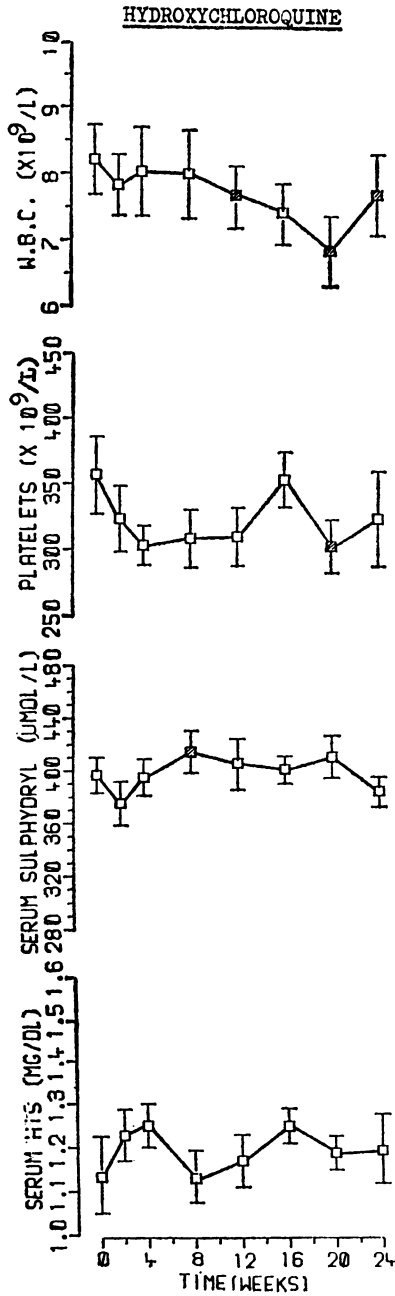
2A

Figs. 2 A, B, C   *Biochemical data (mean ± SE) for rheumatoid patients treated with hydroxychloroquine (left) and sodium aurothiomalate (right). Changes in individual parameters reaching statistical significance (Wilcoxon rank sum test) when compared with data at week 0 are indicated by hatched (p < 0·05) and closed (p < 0·01) data points.*

2B

HYDROXYCHLOROQUINE

GOLD

2C
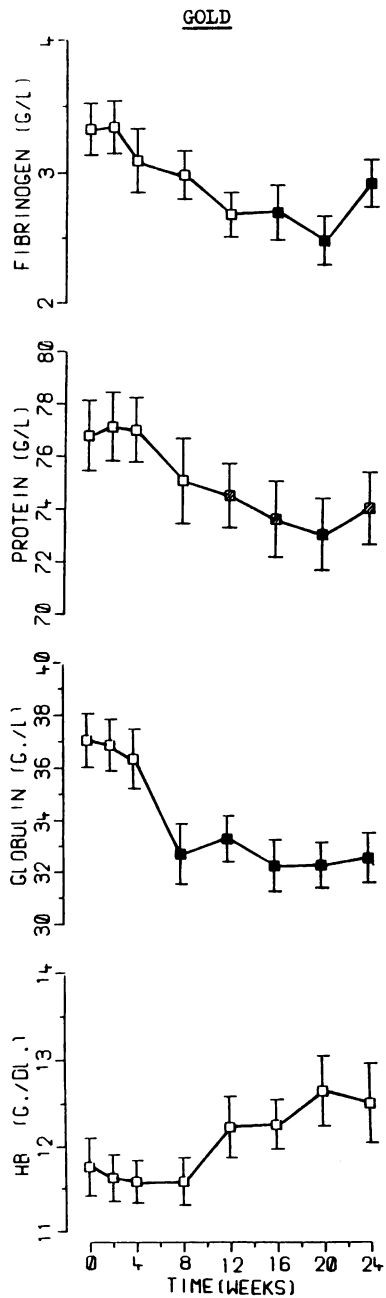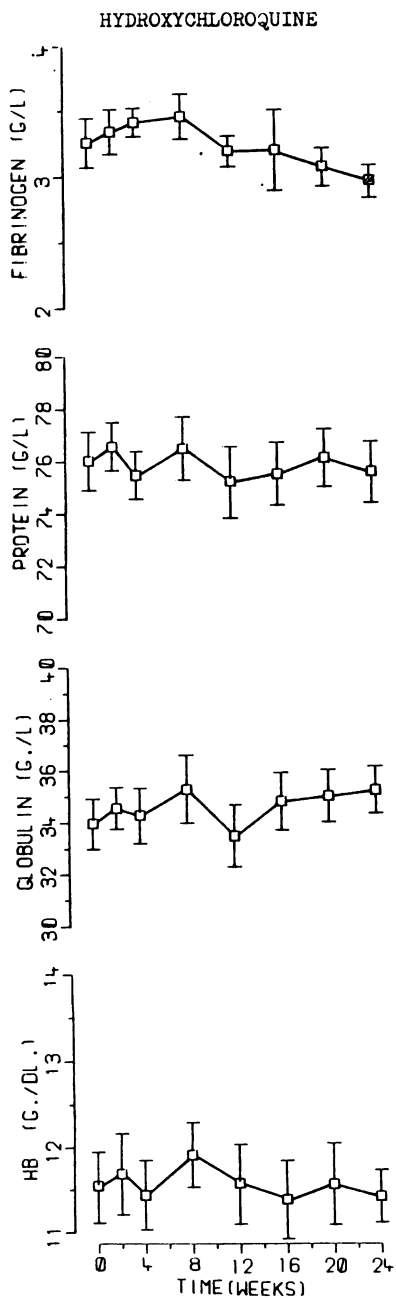
owing to aspirin intolerance. No other drugs were allowed except prednisolone therapy in constant dosage (maximum 7·5 mg.daily) for 2 patients in each drug group.

ASSESSMENT PROCEDURES

Patients were seen at weeks −2, 0, 2, 4, 8, 12, 16, 20, and 24 (0 = date of starting specific antirheumatoid drug therapy). At each visit 18 biochemical and 8 clinical assessments were made as previously described.[1][2]

In view of the reported retinopathy associated with chloroquine and HCQ therapy[3–5] routine ophthalmological tests were performed on the 15 HCQ-treated patients.

All patients completed the 24-week treatment period, irrespective of clinical response, though 2 patients on hydroxychloroquine who failed to attend after week 4 were not replaced.

STATISTICAL METHODS

To test for significant changes in individual clinical or laboratory variables over the 24-week treatment period week 0 data were compared in turn with data from successive clinic visits by the Wilcoxon matched pairs signed rank test for paired data.[6] One-way analysis of variance was used to test for any differences between week 0 data for each variable in the 2 drug groups. Correlation matrices were constructed between clinical and laboratory variables for both HCQ and gold in turn. Each laboratory variable was correlated (Pearson correlation) in turn with each clinical variable; mean data obtained from each of the 8 clinic visits from the start of specific therapy were used.[2]

Results

HYDROXYCHLOROQUINE THERAPY
Of the 13 patients completing 24 weeks' treatment

Table 1   *Correlation matrices for (A) hydroxychloroquine and (B) sodium aurothiomalate. Figures shown represent the significance (p<) of Pearson correlations (r) between mean clinical and mean biochemical variables at successive clinic visits (n=8). Biochemical variables are arranged so that those showing highly significant correlations with clinical variables are placed towards the top*

	Articular index	Joint size	Summated change score	Aspirin dose	Early morning stiffness	Grip strength	Pain score	Functional grade
A. Hydroxychloroquine								
CRP	0·01	0·01	0·001	—	—	—	0·01	0·05
Alkaline phosphatase	0·05	0·01	0·05	0·001	0·01	—	—	—
Plasma viscosity	0·01	0·001	0·001	—	—	—	0·05	—
ESR	0·01	0·05	—	—	—	—	0·05	—
Albumin	0·05	0·01	0·05	—	0·05	—	—	—
Haptoglobin	0·05	0·01	0·01	—	—	—	0·05	—
GGTP	—	—	0·05	—	—	0·05	0·05	—
WBC	—	0·05	0·05	—	—	—	—	—
Fibrinogen	—	0·05	0·01	—	—	—	—	—
SGOT	—	—	—	—	0·05	—	—	—
Platelet count	—	—	—	—	0·05	—	—	—
Bilirubin	—	—	—	—	—	—	—	—
Creatinine	—	—	—	—	—	—	—	—
Globulin	—	—	—	—	—	—	—	—
Protein	—	—	—	—	—	—	—	—
Sulphydryl	—	—	—	—	—	—	—	—
Histidine	—	—	—	—	—	—	—	—
Hb	—	—	—	—	—	—	—	—
B. Sodium aurothiomalate								
Histidine	0·05	0·001	0·001	0·01	0·01	0·01	0·01	0·05
CRP	0·001	0·05	0·001	0·001	0·001	0·001	0·01	—
Sulphydryl	0·01	0·05	0·001	0·001	0·01	0·01	0·001	—
Plasma viscosity	0·01	0·05	0·001	0·01	0·001	0·001	0·01	—
ESR	0·01	0·01	0·001	0·05	0·01	0·001	0·01	—
Haptoglobin	0·01	0·05	0·01	0·01	0·001	0·001	0·01	—
Proteins	0·01	0·05	0·01	0·05	0·01	0·001	0·05	—
Hb	0·05	0·01	0·01	0·05	0·05	0·05	0·05	0·05
Globulin	0·05	—	0·05	0·05	0·01	0·001	0·05	—
Fibrinogen	0·05	—	0·05	—	0·05	0·05	—	—
WBC	—	—	—	—	—	—	—	0·05
Creatinine	—	—	—	—	—	—	—	0·05
Platelet count	—	—	—	—	0·05	0·05	—	—
GGTP	—	—	—	—	—	—	—	0·05
Alkaline phosphatase	—	—	—	—	—	—	—	—
SGOT	—	—	—	—	—	—	—	—
Albumin	—	—	—	—	—	—	—	—
Bilirubin	—	—	—	—	—	—	—	—

GGTP=gamma glutamyl transpeptidase.

Table 2 *One-way analyses of variance to determine the degree of matching between week 0 data for each parameter shown in Figs. 1 and 2 for the 2 drug treatment groups*

Measurement	HCQ		Gold		F	P<
	Mean	±SD	Mean	±SD		
Articular index	21	13	24	13	0·33	NS
Pain score	2·7	0·6	3·1	1·0	1·47	NS
Early morning stiffness (min)	163	186	252	271	0·95	NS
Summated change score	0	0	0	0	0·00	NS
ESR (mm. h^{-1})	49	22	53	21	0·22	NS
Plasma viscosity (cP)	1·92	0·09	1·96	0·17	0·70	NS
Haptoglobin (g.l^{-1})	4·55	1·51	5·14	1·30	1·16	NS
CRP (mg. dl^{-1})	2·60	2·13	4·57	3·36	3·18	NS
Fibrinogen (g.l^{-1})	3·3	0·7	3·3	0·7	0·03	NS
Total protein (g.l^{-1})	76·1	3·9	76·8	4·9	0·18	NS
Globulin (g.l^{-1})	34	3	37	4	4·73	0·05
Haemoglobin (g.dl^{-1})	11·8	1·6	11·8	1·2	<0·01	NS
White cell count (× 10^{9}l^{-1})	8·3	1·9	8·5	2·0	0·06	NS
Platelet count (× 10^{9}l^{-1})	359	108	371	138	0·06	NS
Total serum sulphydryl (μmol.l^{-1})	399	52	377	58	1·06	NS
Serum histidine (mg.dl^{-1})	1·14	0·33	1·16	0·15	0·04	NS

SD=standard deviation.   NS=not significant.

11 were considered to have shown clinical improvement; 2 showed deterioration in all clinical variables measured. All side effects were minor, and there was no retinal damage over the 6-month treatment period.

There was notable improvement in clinical variables by week 12, accompanied by a significant reduction in the acute-phase proteins and plasma viscosity. A downward trend was observed in the erythrocyte sedimentation rate (ESR), though this was not statistically significant. Mean data for clinical and biochemical variables showing a change during treatment are presented graphically (Figs. 1 and 2).

SODIUM AUROTHIOMALATE THERAPY
Thirteen of the 15 patients entered were considered to show clinical improvement; 1 defaulted from the clinic at week 4 and another withdrew with a rash at week 12. Improvement was seen by week 16 as reflected by statistically significant changes in the mean data for clinical variables (Fig. 1) and the acute-phase proteins, ESR, plasma viscosity, serum sulphydryl, serum histidine, globulin, and total protein (Fig. 2). In addition a statistically significant reduction in mean white cell count from baseline values was observed, though in no individual did this necessitate any change in therapeutic regimen. Five patients showed a steady fall in platelet count, also within the normal range, though there was no significant reduction in mean data.

STATISTICAL ASSESSMENTS
Correlation matrices for both HCQ and gold therapy are shown in Table 1A and B respectively. In each matrix the biochemical variables are shown in ranking order, those at the top correlating better with the clinical variables than those at the bottom for that particular drug. Significant correlations were demonstrated between a large number of clinical and laboratory variables as denoted by p levels of less than 0·05. This was particularly noticeable in the gold-treated group, where correlations were in general stronger and more numerous.

The 2 treatment groups were seen to be well matched at week 0 (Table 2), with only globulin showing a significant difference.

Discussion

The use of serum biochemical changes in defining antirheumatoid drug efficacy has been previously established.[1] [7] In terms of the acute-phase proteins, ESR, and plasma viscosity, HCQ produced a later (as defined by the time of significant change in serum biochemistry) and less marked change than did gold or D-penicillamine.[2] This suggests that HCQ, despite its confirmed efficacy in controlled studies in the treatment of RA,[8] [9] is nevertheless less effective than the more established antirheumatoid drugs. The particular value of plasma viscosity in the management of patients receiving hydroxychloroquine is discussed elsewhere.[10]

This finding is substantiated in terms of the correlation matrices constructed between clinical and biochemical variables. Correlations were fewer and weaker for HCQ than for both gold and D-penicillamine.[1] Each matrix was constructed from 144 Pearson correlations each based on 8 data pairs. Hence one can expect 7 falsely significant correlations at p<0·05 (1 in 20) to arise by chance. There were in fact 19 correlations of p<0·05 in the HCQ matrix and 24 in the gold matrix, so about one-third of all

these correlations at p<0·05 were falsely significant. However, this does not detract from our overall finding that the greater improvement in the biochemical and clinical variables for gold-treated patients gave rise to a larger number of significant correlations.

While the acute-phase proteins, ESR, and plasma viscosity[10] have been shown to be non-specific indices of disease improvement, some biochemical variables showed changes which were more specific. For example, serum histidine failed to improve with HCQ therapy[11] but, in contrast to a previous report,[12] showed an upward trend with gold therapy. Haemoglobin levels rose towards normal with gold and D-penicillamine[1] but, in agreement with previous work[9] and in contrast to others,[8] remained unchanged with HCQ. The total serum sulphydryl level improved for gold and D-penicillamine.[13] While this could be attributed to the sulphydryl groups that form part of the structure of these two drugs, we favour an indirect change consequent upon the therapeutic action of these drugs or a combination of both of these as the reason for increased total serum sulphydryl. HCQ's failure to increase serum sulphydryl levels may reflect the drug's weaker activity rather than the lack of sulphydryl group in its structure.

In this parallel group study the analysis showed that week 0 data were essentially matched between the 2 groups, and hence differences in the response of serum histidine, haemoglobin, and serum sulphydryl levels following HCQ therapy compared with gold would suggest a different mode of action of this drug. The failure of total serum sulphydryl, haemoglobin, and histidine levels to improve with HCQ therapy is reflected by the lack of significant correlation between these measurements and the clinical variables.

For the gold-treated patients a temporary rise in the acute-phase reactants was observed at week 2 with the exception of C-reactive protein. This rise achieved statistical significance in the case of ESR (p<0·05). This slight deterioration in biochemical status was also reflected by some other variables such as protein and platelet count, suggesting a genuine initial adverse metabolic reaction to the presence of gold which is rapidly masked by improvement in the disease state. This adds scientific support to the anecdotal clinical impression that some patients experience an initial exacerbation of symptoms on starting gold injections.

Although we favour the greater efficacy of gold as the main explanation for the conflicting results obtained between gold and HCQ, 2 other explanations should be considered. The therapeutic effect may be dose-dependent, and the dose selected is

therefore important in determining results. HCQ might have performed better had we chosen a higher dose than 400 mg/day, though this might have caused a higher risk of ocular side effects. Inequality of patient groups must also be considered in a nonrandomised study. All patients conformed to identical admission criteria and were drawn from the same patient pool, the study being completed within 1 year. We think it unlikely the nature of rheumatoid disease would alter in this short period, and the failure to show a significant difference between patient groups on entry makes a seasonal bias unlikely. Assay of biochemical standards at regular intervals excluded anomalies due to laboratory reagents or technique, and variation in clinical assessment, perhaps the greatest potential source of error, was minimised by intermittent tests of interobserver error between the 2 participating metrologists.

Our findings in this human test system, akin to the testing of drugs at an early stage of their development in an animal model, suggest that HCQ occupies an intermediate position between the more effective drugs, such as gold and D-penicillamine,[2] and weaker drugs, such as alclofenac[2] and aspirin.[14] Controlled clinical trials confirm this ranking order of effectiveness, lending credence to our human test system as a method for detecting antirheumatoid activity in novel compounds.

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