

Putative analgesic activity of repeated oral doses of vitamin E in the treatment of rheumatoid arthritis. Results of a prospective placebo controlled double blind trial

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Abstract

Objective—Vitamin E, the most potent naturally occurring lipid soluble antioxidant has been suggested to possess both anti-inflammatory and analgesic activity in humans. This double blind and randomised study used a broad spectrum of clinical and laboratory parameters to investigate whether there was any additional anti-inflammatory or analgesic effects, or both, of orally administered α -tocopherol in rheumatoid arthritis patients who were already receiving anti-rheumatic drugs.

Methods—Forty two patients were enrolled and treated with α -tocopherol (n=20) at a dose of 600 mg twice a day (2 \times 2 capsules) or with placebo (n=22) for 12 weeks. The following parameters were measured: (1) Three clinical indices of inflammation—the Ritchie articular index, the duration of morning stiffness, and the number of swollen joints; (2) three measures of pain—pain in the morning, pain in the evening, and pain after chosen activity; (3) haematological and biochemical measures of inflammatory activity; (4) assays for the oxidative modification of proteins and lipids.

Results—All laboratory measures of inflammatory activity and oxidative modification were unchanged. Furthermore, the clinical indices of inflammation were not influenced by the treatment. However, the pain parameters were significantly decreased after vitamin E treatment when compared with placebo.

Conclusion—The results provide preliminary evidence that vitamin E may exert a small but significant analgesic activity independent of a peripheral anti-inflammatory effect, but which complements standard anti-inflammatory treatment.

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The clinical symptoms in rheumatoid arthritis (RA) include pain, swelling, and stiffness.¹ Pain sensations arise after stimulation of peripheral nociceptors by inflammatory mediators, which in turn activate specialised sensory pathways. It

is now clear that these pathways are inherently flexible and are subject to extensive modification at both peripheral and central levels. Changes within central pathways play a significant part in the development of pathological pain, including spontaneous pain, secondary hyperalgesia, and allodynia.^{2–4} There is increasing evidence that oxidants contribute to these phenomena by reducing the threshold in the periphery by lowering the threshold of nociceptors or stimulating nociceptor responses, and by facilitating nociceptive transmission within central pathways.^{4,5} Nitric oxide (NO) is one of the free radicals that has been implicated in central pain processing.^{3,6}

Vitamin E (α -tocopherol) is the major lipid soluble antioxidant found in human plasma, erythrocytes, and tissues.^{7,8} The efficacy of vitamin E has been studied in a variety of rheumatological disorders including RA, osteoarthritis, and ankylosing spondylitis.^{9–15} The double blind clinical trials comparing vitamin E with placebo^{13,14} or diclofenac^{9–12,15} were not definitive, but suggested that a dose of 400–1200 mg α -tocopherol daily was effective with respect to various pain parameters such as pain on pressure, pain at rest, and pain on movement. The postulated analgesic properties of vitamin E seem to be correlated to vitamin E plasma concentrations,^{9,10} though it is not known if these are secondary to a peripheral anti-inflammatory action.

If α -tocopherol has a peripheral anti-inflammatory effect this could be related to inhibition of the arachidonic acid pathway^{16,17} or scavenging of free radicals.^{7,8,18} On the other hand, central analgesic effects might be responsible for the pain reduction on treatment with α -tocopherol as vitamin E is known to interact with NO. Nitric oxide, or one of its products, such as peroxynitrite, may react with α -tocopherol, the quinone derivative being a major oxidation product.¹⁹ Depletion of vitamin E by this process could contribute to injury in neuronal tissues. Furthermore, by a mechanism that is independent of its antioxidant properties, *d*- α -tocopherol inhibits protein kinase C,²⁰ a kinase that plays an important part in signal transduction events triggered by neurotransmitters and other cellular stimuli.

A prospective placebo controlled study in patients with RA receiving standard anti-rheumatic treatment was therefore conducted

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to investigate any complementary anti-inflammatory or analgesic effects of α -tocopherol.

Methods

The study was a two centre trial and was of a prospective, double blind, randomised, placebo controlled parallel group design (fig 1). The study was approved by the ethical committees of the The Royal London and Norfolk & Norwich Hospitals and in accordance with the provisions of the Declaration of Helsinki (Hong Kong 1989) and the European Standards of Good Clinical Practice.

Patients with RA as defined by the ARA revised criteria²¹ were enrolled in the study. Inflammatory disease activity was defined as a Ritchie articular index (RAI)²² of at least 6 or early morning stiffness (EMS) lasting at least one hour, or both. Patients had to be between 18 and 80 years of age. They also had to be receiving stable non-steroidal anti-inflammatory drug (NSAID) treatment and 'second line' medication: patients who had had any change in medication, either NSAIDs or second line agents, including corticosteroids, within eight weeks before entering the study were excluded. Those that had been taking vitamin E supplementation or who were vitamin E hypersensitive were also excluded. Patients were allowed to receive intra-articular aspirations with corticosteroid injections, when

clinically indicated, during the trial period: these data were recorded. Further exclusion criteria included pregnancy, malabsorption, and malignancies.

The study design included a run in period of three weeks to ensure that patients fulfilled all inclusion and exclusion criteria. Personal details, past medical history, current treatment, and details of recent intra-articular injections were recorded at the first visit. Joint tenderness was recorded using the RAI. Blood samples were taken for routine and biochemical investigations as listed below.

After completion of the run in period, patients who still met all selection criteria were randomised to receive either 1200 mg *d*- α -tocopheryl acetate as 2 \times 2 capsules daily or visually identical placebo capsules in a double blind fashion for 12 weeks. As no dose response studies have been performed in relation to the effects of α -tocopherol on inflammatory activity in RA patients, the dose was based on the following considerations: (1) The results from controlled trials^{9 10 12-15} in patients with RA and osteoarthritis, which suggested 400–1200 mg of *d*- α -tocopheryl acetate to be effective and well tolerated. (2) The effects of α -tocopherol on pharmacodynamic variables in healthy human subjects. For example, doses of 400–1200 IU/day *d*- α -tocopherol (approximately 270–810 mg/day) for eight weeks were associated with a significant

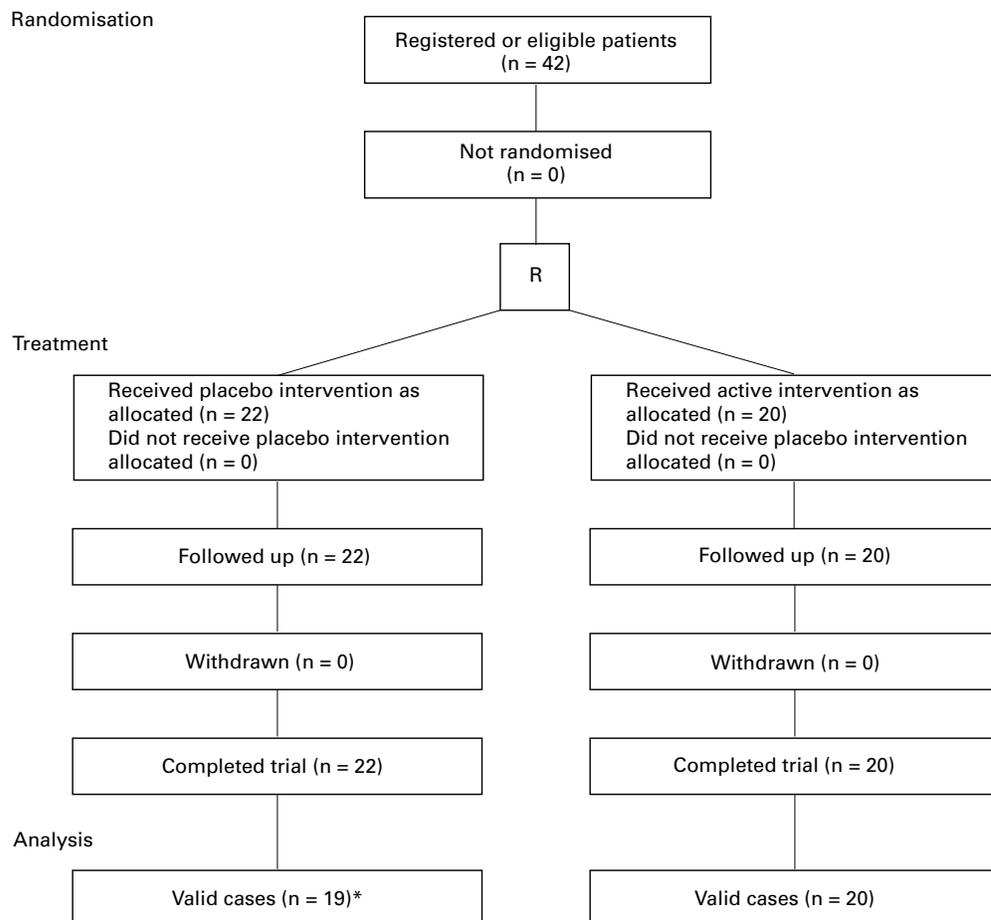


Figure 1 Consort flow diagram. *Inclusion criteria violated by three patients in placebo arm.

Table 1 Baseline characteristics of the total patient population with respect to demographic data and clinical parameters

Baseline characteristics	α -Tocopherol	Placebo	<i>p</i> Value
Patients (n)	20	22	
Age (y; range; mean (SD))	24–75; 55.4 (15.1)	32–66; 52.0 (10.3)	
Sex: female/male	16/4	15/7	
Ritchie articular index (mean (SD))	15.9 (7.7)	14.95 (8.8)	0.702*
Morning stiffness (min; median)	45.0	30.0	0.220†
Number of swollen joints (mean (SD))	9.2 (3.4)	9.8 (5.6)	0.980†
Pain (VAS cm; mean (SD))			
In the morning	4.63 (2.86)	3.74 (2.92)	0.372*
In the evening	4.71 (2.85)	3.99 (3.18)	0.443*
After a chosen activity	5.11 (2.82) (n=19)	4.13 (3.36) (n=21)	0.404*

* = *t* Test, † = U test.

decrease in the susceptibility of plasma low density lipoprotein to oxidation.²³ (3) Preliminary data indicating that the achievable plasma concentrations of orally administered vitamin E were lower in RA patients than in osteoarthritis patients.²⁴ In the current trial, 1200 mg of *d*- α -tocopheryl acetate was chosen to provide an efficacious dose with a high probability of not increasing the overall risk profile of the therapeutic regimen.

Patients continued taking their existing disease modifying, non-steroidal anti-inflammatory and analgesic medication. Clinical evaluation was done after periods of one, four, eight, and 12 weeks of treatment. On each occasion blood was also taken with the exception of week 1. After 12 weeks of treatment patients entered a follow up period of eight weeks. At the final visit (week 20), all study medication having been withdrawn, clinical and laboratory measurements were repeated.

Routine blood tests included a white blood cell count and differential, haemoglobin concentration, mean corpuscular volume, platelet count, and erythrocyte sedimentation rate. Routine biochemistry included electrolytes, urea, creatinine, total protein, albumin, globulin, C reactive protein and rheumatoid factor, urate, total and direct bilirubin, together with alanine transaminase and alkaline phosphatase activities. Specialist investigations included the determination of vitamin E, cholesterol, triglycerides, total lipids, and apoB and apoA-I apolipoproteins. This enabled the vitamin E/lipids ratios to be calculated.²⁵ Thiobarbituric acid reactive substances were measured, using second derivative spectrophotometry (532 nm), as an index of lipid peroxidation.²⁶ The total serum protein carbonyls and the total protein carbonyls/total protein ratio were calculated as an index of serum protein oxidation.²⁷ β Carotene concentrations were meas-

Table 2 Antirheumatic medication including combinations during the trial

	Placebo	α -Tocopherol
Analgesics	10	7
NSAID	18	16
DMARD	14	11
Methotrexate	6	6
Salazopyrin	2	3
Azathioprine	2	1
D-penicillamine	3	0
Myocrisin	0	1
Sulphasalazine	1	0
Corticosteroids	2	1

ured at the same time as vitamin E using high performance liquid chromatography.²⁸

Clinical end points for the assessment of pain considered to be associated with inflammation included the RAI and the duration of EMS. These parameters were supplemented by an assessment of the number of swollen joints. Throughout the study patients also completed a daily diary, which included the duration of EMS and visual analogue scales (VAS) for three different pain parameters. The patients had to mark a 10 cm VAS whose ends were labelled 'no pain' and 'severe pain' with respect to the intensity of morning pain, evening pain, and pain after a chosen activity. The 'chosen activity' was defined by the patient after consultation with the clinical investigator. The chosen activity represented a particular activity repeatedly performed during the day that was causing reproducible pain and that the patient considered to be easily monitored. These three pain parameters, though partially interdependent, were chosen to give a full picture of the patient's pain status because pain intensity in patients with RA varies during the day and with rest or activity. Responders were defined as patients with reduced pain intensity while taking the trial medication. Changes in the patient's general condition, in the concomitant treatment, and the study medication were also recorded. At each visit the investigator and the patient gave their global assessment of efficacy, categorised as 'much better', 'better', 'same', 'worse' or 'much worse'. Treatment compliance was ensured and medication dose checked by pill counting. Adverse events were recorded throughout the study.

STATISTICAL METHODS

The biostatistical evaluation was carried out by means of the statistical software packages SAS and BMDP, the latter in respect of the analysis of covariance with repeated measures.

Statistical analysis included the calculation of median and mean values, minima, maxima, and standard deviations. Standard univariate methods were also used^{29–31}: *t* test for the comparison of mean effects in both treatment groups; *t* test for Pearson's correlation coefficient, *r*; U test for the comparison of median values in both treatment groups; χ^2 test for comparison of frequency distributions in both treatment groups; analysis of covariance with repeated measures for the comparison of time effect profiles in both treatment groups with baseline values as a covariate; two way analysis of covariance. For detection of inhomogeneities concerning anamnestic data or baseline characteristics that might influence the results, multivariate analyses were carried out. A prerequisite for the multivariate analyses is the identification of uncorrelated factors out of the pool of investigated parameters. This factor analysis was done by means of the VARIMAX rotation.³² The principal variable of each identified factor was further analysed in multiple regression and stepwise multiple regression analysis.

Results from previous double blind clinical trials^{9–15} suggested that vitamin E has analgesic

Table 3 Global assessments of change in clinical condition after 12 weeks of treatment with α -tocopherol or placebo (valid case)

Assessment	Patient		Investigator	
	α -Tocopherol	Placebo	α -Tocopherol	Placebo
Improvement	12 (60.0)	6 (31.6)	8 (40.0)	1 (5.3)
No change	4 (20.0)	5 (26.3)	8 (40.0)	11 (57.9)
Deterioration	4 (20.0)	8 (42.1)	4 (20.0)	7 (36.8)

Figures in parentheses are percentages.

and anti-inflammatory properties. Therefore, it was assumed that group differences in favour of vitamin E would occur, and one tailed tests were used to compare the changes from baseline between the two treatment groups. The statistical analysis was conducted both for valid cases—that is, all patients that had participated in the trial according to protocol, and for the intention to treat population—that is, all patients having received the study medication at least once. The level of significance was defined as $p \leq 0.05$.

Sample size calculations were based on anticipated effect differences for the time dependent pain parameters—that is, 'pain in the morning' and 'pain in the evening' using a two sample t test. To detect a 'large' effect of α -tocopherol according to the definition of

Cohen³³—that is, in this study a treatment difference in VAS values of at least $d_t=0.80$, with a power of $1-\beta=0.80$ —20 patients per treatment group were required (one sided t test, $\alpha=0.05$). With respect to multiple end points the level of significance was adapted according to the method described by Bonferroni-Holm³⁴ to obtain the overall level of significance.

Results

A total of 42 patients were enrolled in the study. All patients were suitable for intention to treat analysis and the assessment of drug safety. Three patients in the placebo group had to be excluded from the valid case analysis as they failed the entrance criteria. The valid case population therefore consisted of 20 patients in the α -tocopherol group and 19 patients in the placebo group (fig 1).

Both treatment groups were comparable with respect to baseline characteristics, showing no significant differences (table 1). Fourteen patients in the α -tocopherol group and 16 patients in the placebo group had received intra-articular corticosteroid injections, either more than eight weeks (α -tocopherol: $n=8$, placebo: $n=9$) or within eight weeks (α -tocopherol: $n=3$, placebo: $n=4$) before the start of the study or during the run in period (α -tocopherol: $n=3$, placebo: $n=3$). There were no significant differences in the distribution of concomitant drugs or combination of drugs between the two study groups. The existing anti-rheumatic medication that was continued during the course of the trial consisted of systemic corticosteroids in 7.1%, second line anti-rheumatics in 59.5%, NSAIDs in 80.9%, and simple analgesics in 40.5% in all patients studied (table 2).

Violations of the study protocol were minimal and compliance was assessed to be adequate in all patients. Compliance was confirmed by serum vitamin E concentrations that rose in the tocopherol group from a mean (SD) level of 20.1 (5.6) $\mu\text{mol/l}$ to 43.8 (13.7) $\mu\text{mol/l}$ (a rise of 126.5%) after 12 weeks of active treatment. Vitamin E/serum lipid ratios showed a comparable increase.

Results of the valid case population and of the intention to treat population were very consistent. Therefore, if not stated otherwise, the following results refer to the intention to treat population.

EFFICACY

Assessments of oxidative modification of lipids and proteins, as well as all haematological and biochemical assessments of inflammatory activity were unchanged with α -tocopherol when compared with placebo, with the exception of the concentration of apolipoprotein A-I. Compared with the values obtained with placebo treatment (week 1: 1.37 g/l; week 12: 1.30 g/l), the serum apolipoprotein A-I concentration was significantly higher after α -tocopherol intake (week 1: 1.39 g/l; week 12: 1.42 g/l; $p=0.05$).

The RAI was not significantly influenced by vitamin E in comparison with placebo. With α -tocopherol treatment the mean (SD) value of

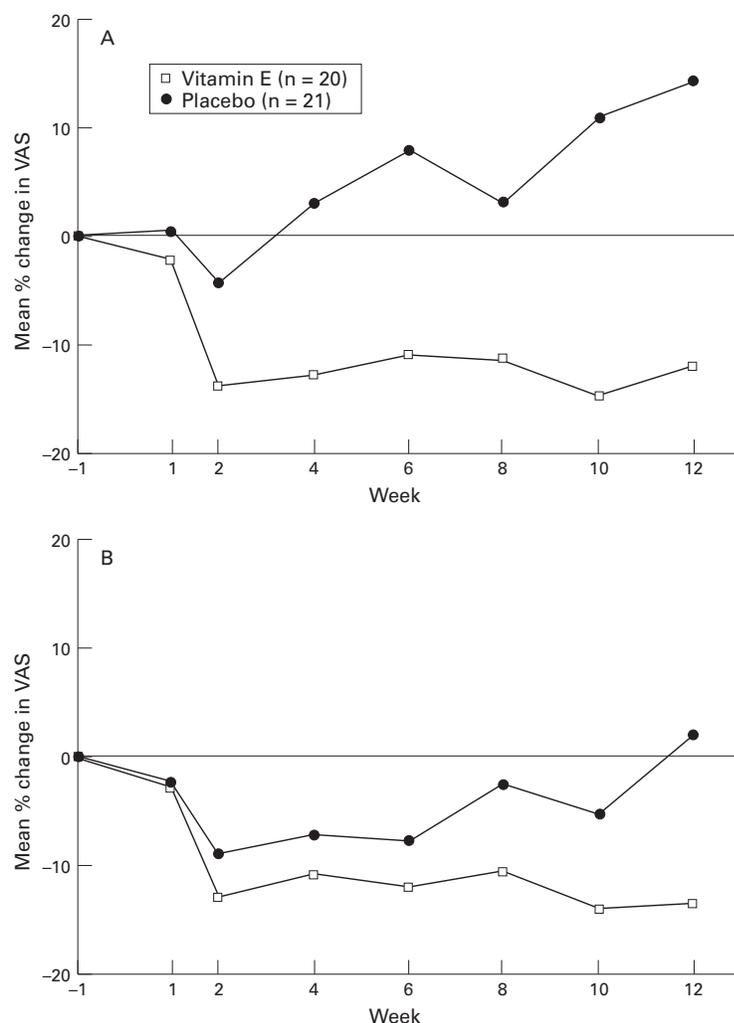


Figure 2 Mean change in the visual analogue scale (VAS) for (A) pain in the morning and (B) pain after chosen activity (intention to treat population).

Table 4 Incidence and nature of adverse events

Adverse events	α -Tocopherol (n=20)	Placebo (n=22)
Skin and appendages disorders	3	–
Central and peripheral nervous system disorders	–	1
Gastrointestinal system disorders	1	2
Urinary system disorders	1	–
General disorder	–	1
Respiratory system disorders	–	3
Total number of events	5	7

the RAI decreased from 15.9 (7.7) to 15.3 (10.0), and with placebo from 14.9 (8.8) to 14.0 (12.1). The median change in duration of EMS (α -tocopherol: 45 min to 30 min, placebo: 30 min to 20 min) and the mean number of swollen joints (α -tocopherol: 9.2 (3.4) to 9.9 (5.0), placebo: 9.8 (5.4) to 10.2 (5.6)) did not show any differences between the treatment groups either.

The mean changes in the three pain parameters, as quantified by self rating VAS, were calculated, as per protocol, as pre-post differences (week 12 minus week 1). The pain parameters significantly decreased with vitamin E treatment when compared with placebo (pain in the morning: -0.56 (1.53) *v* $+0.54$ (1.12), $p=0.006$; pain in the evening: -0.56 (1.43) *v* $+0.28$ (1.00), $p=0.017$; pain after chosen activity: -0.68 (1.52) *v* $+0.09$ (1.19), $p=0.040$). The response rates (week 12 compared with week 1) in respect of these three pain parameters were 55.0%, 50.0%, and 57.9% for vitamin E *v* 22.7%, 27.3%, and 23.8% for placebo (χ^2 test: $p=0.031$, 0.130 and 0.028, respectively). The valid case changes of the pain parameters showed similar differences favouring α -tocopherol. Figure 2 shows the relative changes from baseline. There was no observable difference from baseline until week 2. In the vitamin E group, the analgesic effect is maintained until the end of the treatment. The results of univariate analyses concerning correlations of α -tocopherol treatment and improvement of pain showed that the analgesic effects were not induced by any inhomogeneity. The additional multivariate regression analysis of possible influences of anamnestic or other baseline characteristics confirmed that the changes in pain in the morning ($p=0.011$) and pain in the evening ($p=0.034$) were correlated only with the study medication, favouring α -tocopherol.

According to the patients' global assessment of efficacy, 60% of the patients improved with α -tocopherol compared with 31.8% for placebo. The investigators also favoured α -tocopherol when compared with placebo (table 3).

FOLLOW UP PERIOD

At the end of the follow up period of eight weeks, plasma concentrations of α -tocopherol in the active treatment group dropped to baseline values (22.3 (4.9) mmol/l). After treatment ended all significant differences between vitamin E and placebo with respect to pain or global assessments were lost, and at the end of the

follow up period there were no significant differences between the groups.

TOLERABILITY

No patient showed any remarkable abnormalities in the routine blood tests during the course of the trial. Deviations from normal values were slight, and did not follow a consistent pattern.

The number of adverse events did not differ between the trial groups. Table 4 gives the nature and incidence of adverse events registered in five patients from each trial group. No patient withdrew from the study because of adverse reactions. Reported symptoms were mild and non-specific, and associations with trial drugs were uncertain.

Discussion

It was the aim of this study to investigate whether α -tocopherol has any additional anti-inflammatory or analgesic effects, or both, in patients with RA, when given together with their usual anti-rheumatic medication.

In this study, patients in both groups were comparable with respect to all relevant demographic and baseline features including the existing anti-rheumatic medication. With vitamin E treatment, pain parameters significantly decreased when compared with placebo as shown by the univariate and multivariate regression analyses. The effect was attributable solely to vitamin E and was lost when treatment was stopped.

The superiority of vitamin E over placebo with respect to analgesic effect additional to the existing anti-rheumatic medication was reflected by the global assessments of efficacy. The patients' and the investigators' global assessments favoured vitamin E, showing a trend in the case of the patients' assessments ($p=0.181$) and a significant differentiation between active treatment and placebo in the case of the investigators' assessments ($p=0.035$). In contrast, joint inflammation as assessed by the RAI, the duration of EMS, and the number of swollen joints, was not affected by treatment.

Haematological and biochemical assessments of inflammation were similarly unaffected and none of our specialised investigations of the oxidative damage to lipids and proteins indicated a significant peripheral antioxidant effect.

Our results therefore do not support the view that the observed analgesic effect of 1200 mg *d*- α -tocopherol acetate daily is dependent on a peripheral antioxidant/anti-inflammatory mechanism, but would suggest that the vitamin has a central analgesic action. This has already been proposed in previous human and animal based studies, which indicate that α -tocopherol may act in neuropathic pain syndromes.^{35 36}

Pain is the major feature of RA. However, recent studies have shown that while the normal synovium is richly innervated by free nerve fibres, the innervation is partially lost in patients with RA particularly in the more superficial zones of the synovium.³⁷ In animal

models of arthritis, when injury is induced by a variety of mediators, including oxidants, a similar pattern of denervation occurs.³⁸

Peripheral tissue damage or nerve injury often leads to pathological pain processes, particularly when injury is persistent. The persistence of pathological pain after denervation suggests that changes in central nervous system function play a significant part in pain processing.²⁻⁴ There is considerable evidence to suggest that there is a fundamental interaction of neuropeptides and excitatory amino acids in central nociceptive processing in the dorsal horn neurones.² As already indicated, NO is one of the molecules that modulates this process. In the central nervous system NO is released in response to increases in intracellular Ca²⁺ after activation of receptors for excitatory amino acids. The release of NO stimulates the production of cyclic GMP from soluble guanylate cyclase.^{5,39} Evidence that NO and cGMP contribute to nociceptive processing is given by the finding that nitric oxide synthase inhibitors and inhibitors of soluble guanylate cyclase produce analgesic effects in various nociceptive tests.^{3,40}

The interaction of vitamin E with NO is a mechanism by which it could exert its analgesic effect. Although this interaction is still being elucidated, it is known that there is a loss of α -tocopherol in the presence of SIN-1, a compound that spontaneously decomposes to yield both NO and superoxide.¹⁹ Addition of NO to α -tocopherol results in the loss of vitamin E and quinone formation, showing that NO (or its products) reacts with α -tocopherol. α -Tocopherol also inhibits the activity of NO when assessed as methemoglobin formation in normal and acatalasaemic mouse haemosylates.⁴¹ Nitric oxide synthase gene expression is influenced by the activation of the transcription factor NF- κ B. This activation is partially inhibited by vitamin E.⁴² Furthermore, the hyperalgesia accompanying tissue damage is thought to involve the neurotrophin, nerve growth factor. It has recently been shown that binding of nerve growth factor to the receptor p75^{NTR} results in the activation of NF- κ B, implicating this pathway in the generation of hyperalgesia.⁴³

As mentioned above, we are aware of at least two pertinent previous studies suggesting that α -tocopherol may influence complex neuropathic pain syndromes. In a rat model α -tocopherol influenced the pain response after transection of the sciatic nerve.³⁵ In women with essential dysmenorrhoea there is a rise in β -endorphin-like immunoreactivity in plasma during painful episodes. Intramuscular α -tocopherol rapidly (within minutes) suppressed such pain and was associated with a further rise in β -endorphin-like immunoreactivity.³⁶

In conclusion, pain responses in patients with rheumatoid synovitis are clearly complex, entailing changes within both peripheral and central pathways. Our results show that the administration of α -tocopherol to rheumatoid patients has a small, but significant, analgesic effect that is independent of a peripheral

anti-inflammatory action, thereby suggesting a central rather than peripheral action. This effect might be mediated by a suppressive action on nitric oxide, which has recently been shown to play an important part in central pain processing, or by an inhibitory effect on protein kinase C. These conclusions are limited to patients of similar characteristics to those selected for this study and need to be validated in a larger study population. Furthermore, if α -tocopherol is indeed exerting its analgesic activity centrally, it follows that α -tocopherol induced analgesia will be most effective in reducing those clinical features most strongly associated with central neurogenic plasticity. These features include pain and tenderness referred away from sites of actual tissue damage and the diffuse hyperalgesia seen in many chronic disorders. A number of investigative tools, including quantitative sensory assessments, are now available for use in clinical trials⁴⁴ and future clinical studies might therefore usefully assess the efficacy of α -tocopherol in reducing these disabling features in those chronic arthropathies in which enhancement of central mechanisms would be expected to be greatest. Nevertheless, this study provides interesting data suggesting that α -tocopherol has a central analgesic action in RA. In future studies, it will also be important to establish if this translates into an improvement in a 'quality of life' index.

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