Early report

Effect of topically applied T4 endonuclease V in liposomes on skin cancer in xeroderma pigmentosum: a randomised study

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Summary

Background In patients with xeroderma pigmentosum the frequency of all forms of skin cancer is higher than in the general population, owing to a genetic defect in DNA repair. The bacterial DNA repair enzyme, T4 endonuclease V, delivered intracellularly, increases the rate of repair of sunlight-induced DNA damage in human cells. We tested the ability of this enzyme in a liposomal delivery vehicle applied topically (T4N5 liposome lotion) to lower the rate of new skin cancers in patients with xeroderma pigmentosum.

Methods 30 patients were enrolled in this prospective, multicentre, double-blind study. Patients were randomly assigned T4N5 liposome lotion or a placebo liposome lotion, to be applied daily for 1 year. At 3-monthly visits, new actinic keratoses and basal-cell carcinomas were identified and removed. Analyses were by intention to treat.

Findings 20 patients were assigned T4N5 liposome lotion and ten placebo lotion; one placebo-group patient withdrew before treatment and one withdrew with progressive disease at 9 months. The annualised rate of new actinic keratoses was 8·2 among the patients assigned T4N5 liposome lotion and 25·9 among those assigned placebo (difference 17·7 [95% CI 11·8–26·5]; p=0·004 by Poisson modelling). For basal-cell carcinoma, the annualised rates of new lesions were 3·8 in the treatment group and 5·4 in the placebo group (difference 1·6 [0·38–2·82]). No significant adverse effects were found among any of the patients.

Interpretation DNA damage has an important role in the development of skin cancer and precancerous skin lesions. The topical application of DNA repair enzymes to sun-damaged skin of patients with xeroderma pigmentosum lowered the rate of development of two forms of these lesions during a year of treatment.

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Introduction

Skin cancer is the most common cancer in white people in the USA[1] and other countries. Most skin cancers are basal-cell carcinomas.[2] Early steps in the development of skin cancer are the appearance of ultraviolet-light-induced mutations in the *P53* and *PTCH* tumour-suppressor genes.[3] Actinic keratosis is thought to be a precursor to squamous-cell carcinoma. This dysplastic epidermal lesion is generally discrete and variably erythematous, with an irregular, scaly surface. The prevalence ranges from 25% in temperate countries of the northern hemisphere to 40–60% among adults in Australia.[4] Actinic keratosis is widely believed to be premalignant, because each lesion has a chance of progression to invasive squamous-cell carcinoma of about 8%, and many patients with squamous-cell carcinomas have multiple actinic keratoses.[5] The ultraviolet-light-induced mutations in *P53* that are found in squamous-cell carcinomas are also found in many actinic keratoses.[6]

Patients with xeroderma pigmentosum have an autosomal recessive genetic defect in the pathway that repairs sun-induced damage to DNA.[7] As a result, the rates of malignant disorders of the skin (basal-cell and squamous-cell carcinomas, and the melanocytic disease, melanoma) are 1000 times higher than in the general population, and rates of actinic keratosis are also increased.[8] This defect in DNA repair of damage induced by ultraviolet light has, however, been overcome in cell culture by the intracellular delivery of the bacterial DNA incision repair enzyme T4 endonuclease V.[9]

Treatment of an established basal-cell carcinoma generally consists of Mohs micrographic surgery, surgical excision, curettage and electrodesiccation, or cryosurgery,[10] whereas treatment of actinic keratoses consists principally of observation, cryotherapy, or topical fluorouracil.[11] The only topical treatment to date that has shown efficacy in reducing the risk of actinic keratoses[12,13] is sunscreen, but this requires very careful and constant pretreatment. Oral retinoid therapies are largely experimental and have potentially serious and almost always inconvenient side-effects.[14] T4N5 liposome lotion contains the bacterial enzyme T4 endonuclease V encapsulated in a pH-sensitive engineered liposome for delivery into the living cells of the skin.[15] Topical application of these liposomes in a hydrogel lotion to patients with xeroderma pigmentosum accelerated the removal of ultraviolet-light-induced DNA damage (specifically cyclobutane pyrimidine dimers) compared with placebo, when applied after exposure, and the treatment produced no major adverse effects.[16] In this study, we tested the ability of T4N5 liposome lotion to reduce the rate of appearance of new actinic keratoses and basal-cell carcinomas in patients with xeroderma pigmentosum. Covariates included age, sex, and place of study enrolment (USA or elsewhere).

Methods

Patients

Between July 1996 and January 1998, we recruited 30 patients, aged between 2 years and 65 years, who had a diagnosis of xeroderma pigmentosum confirmed by the unscheduled DNA synthesis assay, and who had a history of actinic keratoses or other skin cancer. The patients were recruited in groups of six. This study had 80% power, at α=0·05, to detect a five-fold difference in the incidence of actinic keratoses. Exclusion criteria were treatment within the previous 30 days with drugs that would interfere with examination of skin lesions, pregnancy, breastfeeding, and inadequate contraceptive measures in women of childbearing potential. In most cases the complementation group (which of the seven possible genes is defective) of the patients was unknown, and the result of the unscheduled DNA synthesis assay alone is not a useful indicator of prognosis. Xeroderma pigmentosum variants are negative in this assay, and patients with such disease would be excluded from this study.

Study design

Four patients in each group of six were randomly assigned T4N5 liposome lotion, and two of the six were assigned placebo lotion. The randomisation code was generated by the Quality Assurance Department at AGI Dermatics by randomisation of six numbers by drawing lots, and the code was not broken until the end of the study on July 20, 2000. The T4N5 liposome lotion consisted of 1 mg/L T4 endonuclease V, encapsulated in liposomes, in a 1% hydrogel lotion. The placebo lotion consisted of equivalent empty liposomes, without enzyme, formulated in the same lotion. The two preparations were indistinguishable by visual examination. Coded bottles were provided by AGI Dermatics, the number matching that assigned to the patient.

Each patient was assessed on a global assessment scale of 1 (mild sensitivity) to 6 (neoplasms) developed by Kraemer and Slor.[17] All actinic keratoses and cancerous lesions were noted and removed by excision or cryotherapy before entry to the study. In addition, all oral or topical drugs that could have interfered with the measurement of new skin cancers or actinic keratoses were discontinued at least 1 month before entry. All patients were instructed on proper daily use of sunscreens of protection factor 15 or greater during the entire study, as well as otherwise maintaining their lifestyles without change. The protocol was approved by the institutional review board of Applied Genetics Incorporated Dermatics, and by each of the ethics committees or review boards governing the clinical investigators.

Each patient received eight containers of 50 mL T4N5 liposome or placebo lotion for each 3-month period of the 1-year study period. They were instructed to apply 4·5 mL to the face and arms daily, as close to noon as possible. The containers were returned at the next visit to the investigating clinician before new containers were issued. At each visit, a blood sample was collected from each patient for laboratory testing. The patient was examined completely, but only lesions on the area of drug treatment (face and arms) were recorded. After the year of lotion use, the patients returned at month 13 and month 18, when any cancers were noted and blood samples were taken.

Analysis

The primary endpoint was the number of new actinic keratoses, and the secondary endpoint was the number of new skin cancers. New lesions on treated sites were identified and recorded for each 3-month period. All new lesions, without a minimum size requirement, were removed at these visits, and the pathology was confirmed histologically, except in the cases of multiple actinic keratoses in the same area. In each study group, the annual rates of new actinic keratoses and basal-cell carcinomas were calculated as the mean of the cumulative total for each patient. When a lesion count was missing, however, the mean and other statistics were calculated based on the available data. For statistical comparisons, the total of actinic keratoses and basal-cell carcinomas for the groups were compared by the Poisson regression model,[13,14] with multivariate analysis (SAS software PROC GENMOD, version 6.12). Age was included as a continuum in this model. Non-significant treatment-by-covariate interactions were dropped from the model by a step-down approach, and non-significant covariate main effects were then eliminated in the same way.

Results

Of the 30 patients with xeroderma pigmentosum who were enrolled, one patient from the placebo group withdrew after randomisation but before treatment was dispensed and was excluded from analysis (figure 1). A second patient from the placebo group withdrew after 9 months because of disease progression.

The two treatment groups were similar in terms of demography and the initial assessment of the severity of disease (table 1). There was no significant difference between the groups in the numbers of skin lesions removed before study entry (p=0·47 for actinic keratoses, p=0·35 for basal-cell carcinomas, p=0·31 for squamous-cell carcinomas, p=0·51 for melanomas by two-sample *t* test), but the time since the previous complete lesion removal for each patient was unknown. For each group, about half of the patients were treated within the USA, and the others were treated elsewhere, predominantly in the UK, Austria, and Germany. All patients used sunscreens of protection factor greater than 15, and all but three used sunscreen of protection factor 30 or more. In a questionnaire, patients reported that, on average,

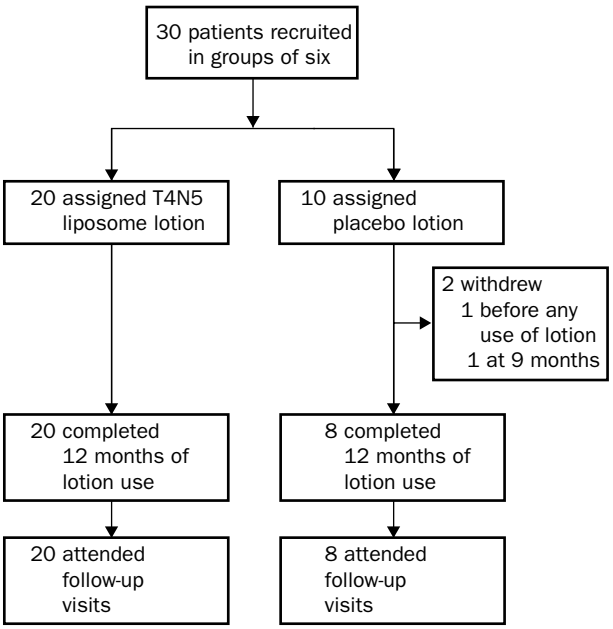

Figure 1: **Trial profile**

Characteristic	T4N5 liposome lotion (n=20)	Placebo lotion (n=9)
Demography		
Median age (range) in years	19·5 (3·5–47)	16·0 (10–53)
Number male/female	4/16	4/5
Number US/non-US	11/9	3/6
Median (range) global assessment*	5 (2–6)	6 (2–6)
Skin type		
I, I/II, II, II/III	14	8
III, IV	6	1

*Of xeroderma pigmentosum: 1=mild sensitivity to 6=neoplasia.[17]

Table 1: **Baseline characteristics of study population**

Outcome	Lesions per year			Estimated coefficient (SE)	p*
	T4N5	Placebo	Difference (95% CI)		
Actinic keratoses	8·2	25·9	17·7 (11·8–26·5)	−2·03 (0·74)	0·004
Age interaction for actinic keratoses					
25th centile (10 years)	1·5	5·8	· ·	· ·	0·007
50th centile (18 years)	2·5	5·5	· ·	· ·	0·04
75th centile (31 years)	5·6	5·4	· ·	· ·	0·893
Basal-cell carcinomas	3·8	5·4	1·6 (0·38–2·8)	−1·05 (0·39)	0·006

*From Poisson regression modelling of 12-month cumulative data.

Table 2: **Rates of new actinic keratoses and basal-cell carcinomas by Poisson modelling**

they did not change their sun-exposure habits during the study. Compliance was good; on average 72·8% of the medication was used, as shown by weighing of the containers returned at each study visit.

The mean number of new actinic keratoses per patient was lower in the group assigned T4N5 liposome lotion than in the placebo group during the first 9 months (figure 2; full data for each patient are given in webtable 3 on www.thelancet.com). In the T4N5 treatment group the rate was similar at 12 months to the 9-month value, but the rate in the placebo group was substantially lower after exclusion of the patient with progressive disease. The range in number of new actinic keratoses during any quarterly visit was 0–32 in the T4N5 liposome lotion group and 0–100 in the placebo group. On a cumulative basis (normalised for a 12-month period), the mean annual rate of new actinic keratoses was 68% lower in the T4N5 liposome group than in the placebo group [(8·2 *vs* 25·9 per patient per year, table 2). This difference was 17·7 lesions per year (95% CI 11·8–26·5; p=0·004, by Poisson modelling).

Poisson modelling also showed a significant treatment effect mediated by age. T4N5 liposome lotion had a significant effect on the rate of new actinic keratoses compared with placebo, in patients younger than 18 years (table 2). For example, the treatment effect at 10 years of age (25th percentile) in the model was greater (p=0·007) than that at 18 years (median, p=0·04), and no significant effect was found at age 31 years (75th percentile, p=0·893). This age interaction with treatment was not attributable to compliance, because there was no correlation between the amount of drug used and age (p=0·77).

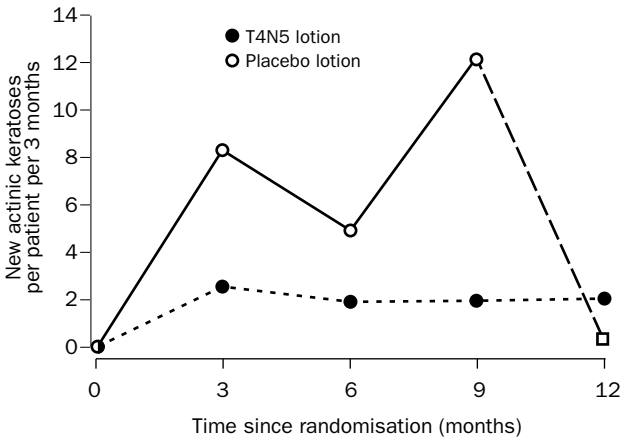

Figure 2: **Rates of new actinic keratoses**
No error bars are shown because the data do not fit the normal distribution assumed for the calculation of SD. The value for the placebo group at 12 months omits the patient who withdrew at 9 months with progressive disease (see webtable 3).

The mean rate of new basal-cell carcinomas was 5·4 per patient per year in the group assigned placebo and 3·8 in the group assigned T4N5 liposome lotion (table 2). The range of new basal-cell carcinomas during any quarterly visit was 0–24 in the T4N5 liposome lotion group and 0–25 in the placebo group (webtable 4). Use of a Poisson regression model showed that the difference in rate of new basal-cell carcinomas per year (1·6 [95% CI 0·38–2·82]), was significant (p=0·006). The covariates of age, sex, and US or non-US study site were not significant. The covariate of basal-cell carcinomas removed before study entry was significantly correlated with the rate of formation of new lesions (p=0·0001).

The rates of development of squamous-cell carcinoma and melanoma in this study population were not large enough for statistical analysis (webtables 5 and 6). There were 15 squamous-cell carcinomas (nine in the 20 treatment-group patients and six in the nine placebo-group patients; annual rate 0·5 *vs* 0·7 per patient; p=0·15 by Poisson modelling). For melanoma there were 13 cancers (11 among the 20 treatment-group patients and two among the nine placebo-group patients; annual rate 0·6 *vs* 0·2 per patient; p=0·15 by Poisson modelling).

Annualised post-treatment rates of actinic keratoses and basal-cell carcinomas did not increase during the 6 months after treatment (webtables 3 and 4).

Some of the patients had higher values in serum chemistry tests and blood counts than hospital norms before entry to the study. The proportion with abnormal results in either study group did not, however, change between baseline measurements and the tests done 3-monthly during the study (data not shown). No patient in either group reported serious side-effects or adverse reactions. No increase in IgG antibodies against the enzyme was detected in serum obtained from all patients at each visit during the study (data not shown). The median response by patients on a graduated 5-step scale, ranging from "unpleasant" to "easy and appealing" was that they found either lotion easy and appealing to use.

Discussion

In this trial, the largest prospective clinical study so far in patients with xeroderma pigmentosum, treatment with T4N5 liposome lotion lowered the rate of new actinic keratoses and basal-cell carcinomas compared with the placebo lotion by 68% and 30%, respectively, during a year of treatment. Because these skin lesions are generally associated with mutations in two different genes (*P53* in actinic keratoses and *PTCH* in basal-cell carcinomas) and the T4 endonuclease V DNA repair enzyme is specific for cyclobutane pyrimidine dimers, these results strongly implicate unrepaired cyclobutane pyrimidine as a common premutagenic lesion in the induction of several forms of skin cancer. Furthermore, the effects on actinic keratoses were observed within the first 3 months of treatment, so the improved repair of DNA damage seems

to affect tumour promotion or progression. The mechanism could be a decrease in immunosuppressive cytokines in skin when the DNA damage is removed.[18] The infertility of the environment in the skin created by a competent immune system is likely to affect the regression of nascent tumours strongly. We expect that over further years of use the protective effect of T4N5 liposome lotion would be even greater.

The treatment effect differed with age; it was significant only in patients younger than 18 years. We should emphasise, however, that xeroderma pigmentosum shortens life expectancy by over 30 years,[8] and therefore patients older than 30 years are a highly selected group. For example, in the placebo group the oldest patient was 53, and the next oldest was 29. Therefore, the age effect is very likely to be a selection effect among ageing patients with xeroderma pigmentosum.

No adverse effects were observed among the patients during treatment, and no antibodies against the enzyme were detected in the patients' serum. This absence of toxicity confirms early safety studies[16] and may be explained by immunohistological observations that T4 endonuclease V delivered by liposomes is localised in the epidermis and does not readily penetrate into dermis.[15] Moreover, during the 6 months after discontinuation of treatment, rates of new actinic keratoses and basal-cell carcinomas did not increase, contrary to experience with retinoids.[14] The lasting effect after the conclusion of the study may imply that T4N5 liposome lotion reverses a fundamental and common source of these neoplasms (cyclobutane pyrimidine dimers[18]), and it suggests that this result is not just a cosmetic effect during the period of application. However, continuing and careful use of T4N5 liposome lotion throughout life is likely to be necessary because further sun damage may accrue.

As rates of skin cancer increase,[19,20] and sunscreens are apparently underused[20] and misused, new drugs are needed that can be used even after sun exposure to reduce skin-cancer formation; such drugs are likely to be of great future value to the normal sun-exposed population, even when sunscreen is used.

Liposome encapsulation of T4 endonuclease V represents a new drug delivery approach that shuttles enzymes across human stratum corneum and introduces biologically active proteins into living epidermis. These clinical results are proof of principle for this enzyme therapy of skin. They suggest that other enzymes and macromolecules could be used in the therapy of other debilitating skin diseases.

Contributors

Daniel Yarosh was responsible for study design, was the principal investigator, and wrote the report. Jonathan Klein was study coordinator and monitor, and reviewed the report. Adrienne O'Connor was director of quality control and assurance and reviewed the report. John Hawk, Elyse Rafal, and Peter Wolf supervised the three largest groups of patients and contributed to discussion and review of the report.

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