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Chemoprevention of Nonmelanoma Skin Cancer: Experience with a Polyphenol from Green Tea

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Abstract

Nonmelanoma skin cancer is extremely common and is increasing in incidence. It would be very useful to have forms of therapy that would prevent precancerous changes from going on to form cancer, or to reverse the precancerous changes. Epidemiologic evidence in humans, in vitro studies on human cells, and clinical experiments in animals have identified polyphenol compounds found in tea to be possibly useful in reducing the incidence of various cancers, including skin cancer. To examine the potential for a polyphenol from green tea, epigallocatechin gallate, to act as a chemopreventive agent for nonmelanoma skin cancer, a randomized, double-blind, placebo-controlled phase II clinical trial of topical epigallocatechin gallate in the prevention of nonmelanoma skin cancer was performed.

Background

Nonmelanoma skin cancer is a significant and increasing medical problem. In the United States, over one million new cases of nonmelanoma skin cancer are diagnosed each year, probably exceeding the incidence of all other types of cancer combined (Jemal et al. 2002). The two most common types of nonmelanoma skin cancer are basal cell carcinoma and squamous cell carcinoma. The greatest risk factors for these types of cancers are a fair skin type and high cumulative exposure to sunlight.

The most readily identifiable premalignant lesion for nonmelanoma skin cancers is actinic keratosis (solar keratosis), which is considered to be a premalignant precursor to squamous cell carcinoma. There is some controversy over this designation among dermatologists, some of whom consider actinic keratoses to be a form of squamous cell carcinoma in situ of the skin (Heaphy and Ackerman 2000). The risk factors for actinic keratoses are the same as

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those for nonmelanoma skin cancer, as described above. Given these risk factors, it is not surprising that fair-skinned populations inhabiting sunny climates at latitudes closer to the equator such as Australia have the highest risk for, and incidence of, actinic keratoses (Marks 1990).

Actinic keratoses are readily identifiable precursors to nonmelanoma skin cancer; as a result they are useful clinical markers for cancer prevention research. Interventions that lead to the regression or decrease in the rate of formation of actinic keratoses would be expected to lead to a decrease in the formation of nonmelanoma skin cancers, particularly squamous cell carcinoma of the skin. It is for this reason that actinic keratoses are being extensively studied as modulable clinical endpoints in trials testing the chemopreventive potential of promising agents against the development of nonmelanoma skin cancer.

The purpose of this chapter is to discuss important aspects of study design for clinical trials of potential chemopreventive agents administered to prevent, regress, or retard the development of actinic keratoses and nonmelanoma skin cancer. Our experiences with a polyphenol from green tea will be used as an example.

Choice of Potential Chemopreventive Agents for Actinic Keratoses and Nonmelanoma Skin Cancer

In choosing a candidate chemopreventive agent for nonmelanoma skin cancer, several factors must be considered. The factors include epidemiologic evidence indicating that the chemopreventive agent may be useful in the prevention of the cancer of interest and observational data on drugs developed for other applications (e.g., oncologic agents such as topical 5-fluorouracil). Activity of the potential chemopreventive agent in animal model systems is an important form of preliminary evidence. Additionally, in vitro activity against precancerous and cancerous cells may be of use. If there is a potential molecular mode of action postulated for the agent, various forms of biochemical and molecular studies may be used to evaluate a potential chemopreventive agent as well. In our study of the chemoprevention of actinic keratoses and squamous cell carcinoma, epigallocatechin gallate satisfied many of the above-mentioned criteria for advancement into clinical testing.

Epidemiologic evidence suggests that green tea may have anticancer activity against a number of cancers. However, the evidence is equivocal and various studies in different populations show that green tea may be associated with a preventive effect, no effect, or even an increased risk of cancer in nearly every epithelial cancer studied (see Bushman 1998 for review). In addition to differences in study design, plant variety, growth conditions, horticultural practices, processing, and variable tea consumption may contribute to the mixed data arising from epidemiologic studies (Katiyar and Mukhtar 1997). The bulk of the epidemiologic evidence probably does favor some chemopreventive effects for certain cancers. Animal model studies of epigallocatechin

gallate, a tea constituent, have demonstrated inhibitory effects against carcinogenesis at a number of organ sites. Particularly convincing preliminary evidence is that the compound has been shown to reverse squamous papilloma formation in the mouse model of UV carcinogenesis that is most analogous to actinic keratoses in humans (Mukhtar et al. 1994; Gensler et al. 1996). There is also in vitro evidence using human skin cancer lines that epigallocatechin gallate may possess anticancer properties (Valcic et al. 1996). Numerous molecular mechanisms have been suggested to account for the possible chemopreventive activity of green tea against various cancers. The polyphenols, including epigallocatechin gallate, are powerful antioxidants that may act to quench free radicals produced during the carcinogenic process; also, they may act at many sites in the carcinogenic pathway (Dreosti 1997). Given the epidemiologic, animal model, in vitro, and molecular evidence, it was felt that epigallocatechin gallate was a good candidate agent for chemoprevention of human nonmelanoma skin cancer and a clinical trial was designed to test this hypothesis.

Another consideration is that any new chemopreventive agent will be judged against current therapies, which for actinic keratoses mainly consist of liquid nitrogen treatment, treatment with the topical chemopreventive agent 5-fluorouracil, photodynamic therapy (Jeffes et al. 2001), and more recently, treatment with the topical agent diclofenac (Rivers et al. 2002).

Clinical Trial Design for Chemoprevention of Nonmelanoma Skin Cancer

There are several important elements that must be included in a clinical trial of potential chemopreventive agents for actinic keratoses and nonmelanoma skin cancer. The trial must include a control arm. This is essential because actinic keratoses and visual signs of photodamage are usually graded and monitored clinically, which introduces a large element of subjectivity. For example, the application of emollient type vehicles can appear to regress actinic keratoses and photodamage simply by inducing immediate surface changes. For these reasons, it is necessary that the study be double-blinded, such that neither study participants nor investigators know whether the area under evaluation is being treated with the study agent or the placebo. Even for oral agents, the inherent subjectivity of clinical grading systems mandates inclusion of a control group and randomized, blinded studies.

Study Population Considerations

In regards to the population at risk and to be studied, the study cohort will primarily consist of fairer skinned individuals with a considerable amount of sun exposure. This translates into the need to recruit subjects mainly in their 60s or older, though rarely people even as young as those in their late 20s may have enough sun damage and actinic keratoses to qualify as study subjects.

Study subjects can be referred from associated dermatology clinics or recruited directly from the community through directed advertising. For our study of a topical green tea polyphenol, study participants were recruited from associated university and private dermatology clinics, and by various forms of print and radio advertising, which targeted the age demographics of the eligible study participants.

Design of Treatment Methodologies in Skin Cancer Chemoprevention Studies

Treatment methodology will depend on whether the agent is topical or oral. For topical formulations, study subjects can often serve as their own controls, with one body part designated as a target area to be treated with the active agent and the mirror area on the opposite side of the body receiving the placebo control. Again, the sides to receive the treatment and the control should be assigned in a random and double-blinded fashion. For clinical trials with oral agents, each participant must be randomized to active treatment or control, though there does not need to be an equal number of treatment and control subjects – the control arm can consist of a smaller number of study subjects. Statistical analysis will determine the proportion of subjects that should be allotted to the control group so that the study achieves an acceptable balance between statistical power and the desire of subjects to receive potentially active treatment rather than placebo.

For our trial, epigallocatechin gallate was compounded in a topical vehicle to a final assayed concentration of 5.5–8.5%, 0.5 ml was applied by the subject nightly for 12 weeks to target actinic keratoses on one of the forearms. A matched placebo ointment was applied to target actinic keratoses on the opposite forearm. Prior to treatment, at least two actinic keratoses were identified on each forearm target area, and these target actinic keratoses were mapped, followed, and clinically graded at 2-week intervals during the 12-week treatment period.

Statistical Considerations in Treatment Methodology Design

It is necessary to design the study such that it will have adequate power to detect the hypothesized differences between the agent and the control group for the parameters under study.

For our study with epigallocatechin gallate, 51 participants were randomized to either active agent or placebo, which provided more than adequate power to detect a 50% difference between active and placebo groups. It was felt that this level of activity would be necessary for the agent to be promising for future studies and possible development.

Surrogate Endpoint Biomarker Analyses

If possible, in addition to monitoring the clinical status of actinic keratoses during the trial of chemopreventive agents, it is desirable to include analyses of surrogate endpoint biomarkers for the actinic keratoses and the squamous cell carcinomas into which they may evolve. The elucidation of useful surrogate endpoint biomarkers is at an early stage for nonmelanoma skin cancer. The most likely candidate surrogate endpoint biomarker for actinic keratoses and squamous cell carcinoma is p53 (Brash et al. 1991; Einspahr et al. 1997). Good candidate surrogate endpoint biomarkers have yet to be identified for basal cell carcinoma.

For our study, we tried to choose a variety of potential surrogate endpoint biomarkers, with a goal being the evaluation of the usefulness of these biomarkers in chemoprevention studies for actinic keratoses and nonmelanoma skin cancer. The biomarkers selected were: (1) p53, a cell cycle regulatory protein; (2) Ki-67, a marker of proliferation; (3) CD1A, a Langerhans cell marker – Langerhans cells being the main immune surveillance cells of the skin; (4) nucleolar number; and (5) various measures of nuclear morphometry, including nuclear size and shape and variability in size and shape.

We chose these markers because we felt that they have good potential to be useful surrogate endpoint biomarkers for actinic keratoses and nonmelanoma skin cancer. They represent a wide variety of categories of possible biomarkers including cell cycle regulation, cell proliferation, and cellular morphology, and because all of these biomarkers can be measured quantitatively in an objective manner with computer-assisted image analysis.

Skin biopsies were performed on actinic keratoses, sun-damaged skin, and nonsun-damaged skin pretreatment. Also, skin biopsies were performed on actinic keratoses and sun-damaged skin in the areas that agent or placebo was applied to posttreatment as well. This was done for histopathologic analysis of the changes and surrogate endpoint biomarker analysis between the different groups represented by the biopsies.

Results of Our Study Using Topical Epigallocatechin Gallate as a Chemopreventive Agent for Actinic Keratoses and Nonmelanoma Skin Cancer

A total of 51 study subjects completed the 12-week study, wherein the agent was applied to one forearm with actinic keratoses and placebo ointment to the other forearm nightly. Analysis of the clinical monitoring of the target actinic keratoses at 2-week intervals revealed a slight, progressive decrease in grade of severity of the actinic keratoses over the course of the study in both the treatment and control groups. There were no statistically significant differences between the treatment and control groups. This illustrates the necessity

of including a control group, without which we might have concluded that the agent showed efficacy in the treatment of actinic keratoses.

Analysis of the surrogate endpoint biomarkers is ongoing; however, analyses have been completed on the majority of the biomarkers. For those completed, there was no significant difference between the treatment and control groups. However, for all biomarkers analyzed to date except CD1A, there are statistically significant differences between biomarkers between actinic keratoses and sun-damaged skin and actinic keratoses and nonsun-damaged skin. There were nonstatistically significant differences for the surrogate endpoint biomarkers so far analyzed between sun-damaged and nonsun-damaged skin.

Discussion of Results of Topical Epigallocatechin Gallate as a Chemopreventive Agent in Nonmelanoma Skin Cancer

Preliminary analysis of the clinical and surrogate endpoint biomarker data indicates that topical epigallocatechin gallate is not active in the formulation used in our study. There are several possible explanations for this. Epigallocatechin gallate may simply not be an effective clinical agent for actinic keratoses/nonmelanoma skin cancer. This is certainly a possibility, but one that is not easy to reconcile with the impressive animal model data (Mukhtar et al. 1994; Gensler et al. 1996). Another possibility is that the particular formulation used was not effective. Reasons for this could be that the active compound, epigallocatechin gallate, is not stable in the current formulation, or that the compound is stable, but is not being delivered in an effective manner to the target site in the skin tissue. The epigallocatechin gallate formulation used in this study has undergone repeated stability testing throughout the course of the study and shows minimal degradation over the time frame in question. As a result, compound instability is not likely to account for the inactivity of this formulation. Questions about the bioavailability of the specific formulation used and possible design of a more effective formulation are areas that merit further study.

Although the current study did not demonstrate agent efficacy, surrogate endpoint biomarker analysis is yielding useful data that tentatively support the biomarkers chosen (with the exception of CD1A) as having potential in differentiating sun-damaged from precancerous and cancerous states.

Further conclusions await complete analysis of the data.

Summary

Nonmelanoma skin cancer constitutes a significant human disease burden and new methods of prevention need to be developed. Given the limited success of primary behavioral prevention, there is a strong need for chemopreventive agents against nonmelanoma skin cancer. Our study of epigallocate-

chin gallate for the treatment of actinic keratoses has shown minimal to no clinical activity; however, this might be attributable to the particular formulation used. Further data analysis and experiments are underway to better understand these findings. The surrogate endpoint biomarker analysis, which showed differences between actinic keratoses and sun-damaged skin, has yielded promising information that will provide insights into biomarkers for skin carcinogenesis and potentially help identify surrogate endpoints that may prove useful in the design and interpretation of future clinical trials.

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References

Brash DE, Rudolph JA, Simon JA, et al (1991) A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc Natl Acad Sci USA 88:10124–10128

Bushman JL (1998) Green tea and cancer in humans: a review of the literature. Nutr Cancer 31:151–159

Dreosti IE (1997) Cancer biomarkers in the field of tea. Cancer Lett 114:319–321

Einspahr J, Alberts DS, Aickin M, et al (1997) Expression of p53 protein in actinic keratosis, adjacent, normal-appearing, and nonsun-exposed human skin. Cancer Epidemiol Biomarkers Prev 6:583–587

Gensler HL, Timmermann BN, Vlacic S, et al (1996) Prevention of photocarcinogenesis by topical administration of pure epigallocatechin gallate isolated from green tea. Nutr Cancer 26:325–335

Heaphy MR Jr, Ackerman AB (2000) The nature of solar keratosis: a critical review in historical perspective. J Am Acad Dermatol 43:138–50

Jeffes EW, McCullough JL, Weinstein GD, et al (2001) Photodynamic therapy of actinic keratoses with topical aminolevulinic acid hydrochloride and fluorescent blue light. J Am Acad Dermatol 45:96–104

Jemal A, Thomas A, Murray T, et al (2002) Cancer statistics, 2002. CA Cancer J Clin 52:23–47

Katiyar SK, Mukhtar H (1997) Tea antioxidants in cancer chemoprevention. J Cell Biochem Suppl 27:59–67

Marks R (1990) Solar keratoses. Br J Dermatol 122:49–54

Mukhtar H, Katiyar SK, Agarwal R, et al (1994) Green tea and skin-anticarcinogenic effects. J Invest Dermatol 102:3–7

Rivers JK, Arlette J, Shear N, et al (2002) Topical treatment of actinic keratoses with 3.0% diclofenac in 2.5% hyaluronan gel. Br J Dermatol 146:94–100

Valcic S, Timmermann BN, Alberts DS, et al (1996) Inhibitory effect of six green tea catechins and caffeine on the growth of four selected human tumor cell lines. Anticancer Drugs 7:461–468