

DNA REPAIR ADJUVANTS

THE ROLE OF AC-11, LIPOSOME LOTION
DIMERICINE AND SELENIUM IN ENHANCING
HEALTH AND PREVENTING SKIN CANCER.

BY NOAH SCHEINFELD M.D., J.D.

While anti-oxidants have long been getting a great deal of attention as prophylactics of and panaceas for cancers, there's a new group of therapeutic compounds in the spotlight — DNA repair promoters. So how exactly do DNA repair promoters and compounds promote the repair of human DNA and what is their role in the enhancement of human health?

BACKGROUND ON DNA REPAIR

A cause of cancer is errors in DNA sequences that manifest in newly replicated DNA. There are two main types of errors: (1) dimers in the form of C → T, DNA and (2) DNA mismatch repair. In normal skin, DNA repair or correction of these dimers and corrupted DNA requires coordination of more than 20 proteins, which together can repair approximately 50 percent of damaged DNA incurred daily.

Ultraviolet radiation causes DNA damage by producing cyclobutane pyrimidine dimers (CPD). Cytosine to thymidine (C to T) transition mutations result, and serve as "molecular finger-

prints" for UVB-damaged DNA. These dimers are a common cause of mutations in DNA.

Another type of DNA mutation is caused by stochastic changes in DNA during replication. These errors are constantly corrected by DNA repair proteins, which are referred to as the DNA mismatch repair system of the cell. This machinery detects and corrects errors in DNA replication in which the wrong DNA unit is stitched into place in a newly forming DNA strand. Normally such units — called nucleotides — on one strand of the double-stranded DNA molecule bond with complementary nucleotides on the other strand, like complementary pieces of a puzzle. Thus, an adenine on one strand is normally paired with a thymine on the other, and a guanine on one strand with a cytosine on another.

The process of mismatch repair involves first recognizing the mismatch — for example of an adenine with a cytosine. The machinery then recognizes a break in the newly synthesized DNA strand, which triggers the machinery to excise the section including the mismatch, starting at the strand break and working toward the mismatch and slightly beyond. The system then replaces the mismatched strand with one containing the correct complementary nucleotide. The power of the DNA repair machinery is highlighted by the fact that the mismatch repair system is flexible enough to recognize such a triggering strand break on either side of the mismatch along the DNA strand.

DNA REPAIR ADJUVANTS

DNA repair promoters or adjuvants can be divided into three categories (1) minerals (e.g. selenium and selenomethionine), (2) carboxyl alkyl esters i.e. AC-11 (formerly known as C-MED-100 derived from Cat's Claw [*Uncaria tomentosa*]) and (3) Dimericine, a DNA repair enzyme, (T4 endonuclease V) encased in liposomes.

Selenium and AC-11 can be taken orally. AC-11 and Dimericine can be applied topically alone or in combination with other agents — most commonly a sunblock. Of the three DNA repair enhancing agents discussed, only AC-11

RECENT DISCOVERIES IN DNA REPAIR

The mechanisms that underlie DNA repair have been extensively explicated in recent years. A few recent discoveries in the field DNA repair enzymes follow.

1. **Modrich**¹ found that a protein called PCNA is clamped onto the DNA at the strand break. PCNA, together with the protein that clamps PCNA onto the DNA double helix, regulate the enzyme whose job it is to snip out the segment containing the mismatch, by "aiming" the enzyme — known as exonuclease I — in the right direction to work itself along the strand, stripping out the segment containing the mismatch. A notable aspect of this PCNA repair system is that it can evaluate the placement of the strand signal to one side or the other of the mismatch and work from there. Placement of the strand break that directs repair to one side or the other of the mismatch might be due to of mechanism by which DNA is copied by the replication machinery.

2. **Ronai**² found that the protein ATF2 (Activating Transcription Factor-2) is activated by a protein kinase called ATM (Ataxia-Telangiectasia Mutated), which stimulates DNA repair. ATF2's role in regulating expression of proteins that control cell cycle and programmed cell death is well established. Ronai demonstrated ATF2's role in DNA repair, an intracellular process that prevents formation of genetic mutations, including those that lead to cancer. ATF2 is regulated by ATM and this regulation is central to the cell's ability to initiate DNA repair processes following ionizing irradiation or other exposures that cause breaks in DNA. ATF2 likely works by halting the cell's cycle to allow repair of damaged DNA before such damage becomes permanent.

3. **Powell**³ discovered that MDC1, a protein previously recognized only for its function in sensing DNA damage and signaling its presence, also transports DNA-repair proteins to the site of DNA strand breaks. Without MDC1 to pave the way, repair happens slowly because the fix-it proteins have a hard time reaching damaged areas, which are buried in the tightly packed chromosomal material of the cell's nucleus. MDC1 can bind to chromatin, the complex mixture of DNA and proteins that holds the genetic material. Because of chromatin's properties, getting into it to reach the DNA strand requires the right 'passwords.' MDC1 provides the DNA-repair proteins with this privileged access, and efficiently transports them to the site of damage so they can effect repair. ■

can be administered both orally and topically. The DNA repair adjuvants will be discussed herein.

SELENIUM

Selenium has been touted as a substance that can prevent or treat cancer. There are two possible mechanisms for this: antioxidant effects or enhancement of DNA repair.

It was noted by Seo⁴ that selenium in the form of selenomethionine (SeMet) can activate the p53 tumor suppressor protein by a redox mechanism that requires the redox factor Ref-1. Specifically, using ultraviolet light to

damage cellular DNA, the research team found that treatment of the cells with selenomethionine activated a protein called Ref-1 that switched on p53 causing a three-fold increase in p53 activity and a doubling of DNA repair in the cells. As a result, cells with functional p53 can tolerate higher doses of ultraviolet light if grown and maintained in the presence of selenomethionine. Rafferty, more recently in 2003, however, concluded that selenite and selenomethionine protect keratinocytes from UVR-induced oxidative damage, but not through creation or formation of UVR-induced excision repair sites.⁵ So while

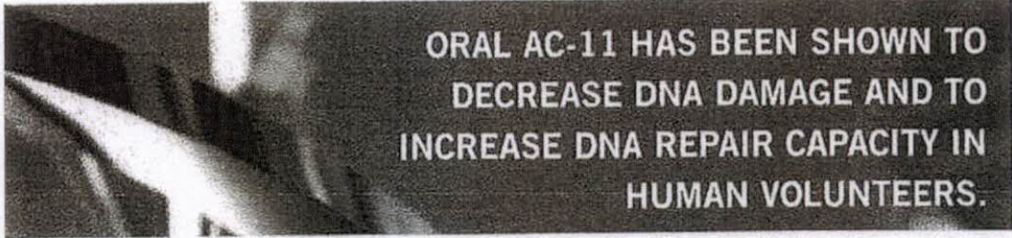
selenium might prevent cancer, it might not be acting to do this by promoting DNA repair.

AC-11

Oral AC-11 has been shown to decrease DNA damage and to increase

nine and strand breaks) is possibly due to enhanced base excision repair or an inherent antioxidant effect, or both. Repair of double-strand breaks suggest an end-joining mechanism. It's possible AC-11 activates/augments histone acetyltransferases, but it's pure speculation.

pilot study that included 42 healthy volunteers there were dramatic and significant ($p < 0.0001$) reductions in erythema and blistering in volunteers who applied 0.5% topical AC-11 with and SPF-15 sunscreen when compared to the group who just applied an SPF-15 sunscreen.



ORAL AC-11 HAS BEEN SHOWN TO DECREASE DNA DAMAGE AND TO INCREASE DNA REPAIR CAPACITY IN HUMAN VOLUNTEERS.

DNA repair capacity in human volunteers. Immune enhancement was observed in response to pneumococcal vaccine in volunteers given oral AC-11 and an *in vitro* study demonstrated that AC-11 inhibits the nuclear transcription factor, NF- κ B, which is activated in inflammatory diseases such as arthritis, asthma and inflammatory bowel diseases.^{6,9}

AC-11, a water-soluble extract of *Uncaria tomentosa*, has been noted effective in repairing both oxidative (8-hydroxyguanine, single- and double-strand DNA breaks) and photochemical (cyclobutyl pyrimidine dimers) DNA damage. *Uncaria tomentosa* grows in the Amazon basin and has been used historically by indigenous tribes for medicinal purposes. The carboxyl alkyl esters in the Cat's Claw extract found in AC-11 are the main components thought to be responsible for enhancing the repair of DNA. The recommended oral dose is 350 mg/day. AC-11 is applied topically in a 0.5% concentration.

THE ROLE OF AC-11 IN DNA REPAIR

While no data exists to support that contention of a beneficial effect on mismatch repair for AC-11, it's possible AC-11 does exert such a role. AC-11 appears to enhance the normal repair of cyclobutyl pyrimidine dimers following UV-B exposure. We can infer, with some confidence, that AC-11 enhances nucleotide excision repair (or photoreactivation). The observed reduction in oxidative DNA damage (8-hydroxygua-

Reduced non-melanoma skin cancer following topical application of AC-11 in hairless mice (an unpublished study) is likely due to a reduced dimer burden. Decreased dimers – decreased *p53* mutations – decreased actinic keratosis – decreased malignancies.

MECHANISM OF AC-11

The mechanism for AC-11 activity has yet to be fully defined; however, research in humans and in human living skin equivalents shows that AC-11 reduces erythema and blistering after ultraviolet exposure.^{10,11} AC-11 significantly enhanced the repair, but not the formation, of cyclobutyl pyrimidine dimers (TT-dimers) in human living skin equivalents exposed to UV-B light.

In a study of 5 healthy volunteers aged 35 to 55 year old taking 350 mg/day of AC-11 orally for 4 weeks, 8-hydroxyguanine levels were significantly ($p < 0.05$) decreased.⁶ The beneficial effect was noted to persist two weeks after therapy was discontinued. Another study reported a significant ($p < 0.05$) decrease in DNA single-strand breaks following peroxide-induced DNA damage in monocytes of healthy volunteers who received 8 weeks of AC-11 at 350 mg/day.⁷

Pero et al, assessed oxidative DNA damage in 14 volunteers, most of which (more than 75%) had chronic diseases, and reported that 9 of the 14 volunteers had decreased 8-hydroxyguanine DNA adducts after 400 mg of AC-11 per day for 4 weeks. Finally, in a in a single-blind, right side-left side, beach sun exposure

AC-11 AND ANIMAL STUDIES

The DNA data in humans has been supplemented with two animal studies in which the effects of known DNA damaging agents were compared in AC-11-treated and control animals.

In the first study,⁸ daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered to 20 rats (an additional 10 rats served as controls) by gavage for 8 weeks. Half the animals from each group were exposed to 12 Gy whole body radiation (¹³⁷Cs source) and allowed 3 hours to repair *in vivo* before DNA damage was assessed. AC-11-treated animals almost completely repaired single-strand DNA breaks ($p < 0.05$) for both AC-11 doses compared to untreated animals. Double-strand DNA breaks were substantially less in animals treated with 40 mg/kg/day of AC-11 and significantly ($p < 0.05$) less in animals treated with 80 mg/kg/day of AC-11 compared to untreated animals.

In the second study,⁹ daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered orally to 8 rats (4 at each dose) 24 hours after the last of three 2 mg/kg intraperitoneal doses of doxorubicin. Four animals received doxorubicin only. Animals treated with 80 mg/kg of AC-11 had significantly ($p < 0.05$) reduced DNA damage in the form of single-strand DNA breaks.

More recently Pero¹² reported on the combination of a Cat's Claw water extract (AC-11, carboxy alkyl esters = active ingredients) plus medicinal mushroom extracts (*Cordyceps sinensis*, *Grifola blazei*, *Grifola frondosa*, *Trametes versicolor* and *Ganoderma lucidum*, polysaccharides = active ingredients) plus nicotinamide plus zinc into a formulation designed to optimize different modes of immunostimulatory action in 14 subjects treated for 4 weeks and found patient experienced reduced pain, reduced fatigue, weight loss and a reduced presence of DNA damage in peripheral blood assessed by (8-OH)

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guanine DNA adducts and elevation in serum protein thiols.

DIMERICINE

T4 endonuclease V (Dimericine) is a DNA repair enzyme produced in bacteria that is delivered in liposomes in the

ious intervals after controlled UV exposure. Biopsies conducted 6 hours after UV exposure revealed that patients with XP had achieved approximately 15% fewer CPDs (improved DNA repair) while patients with a history of skin cancer achieved less than 10% fewer CPDs.¹⁵

skin cancer. DNA repair adjuvants appear to be promising health aids. AC-11 and Dimericine appear to be best used in conjunction with other agents to optimize their health promoting utility. While promising, the ultimate clinical effectiveness and effect on outcome, mode of administration and mode of utilization with other agents remains to be fully defined. ■

Dr. Scheinfeld is currently an alternate of the Investigational Review Board at St. Luke's-Roosevelt Hospital in New York City.

Disclosure: Noah Scheinfeld is a consultant for Optigenex, the maker of AC-11.

DNA REPAIR ADJUVANTS APPEAR TO BE PROMISING FOR ENHANCING HEALTH AND RETARDING THE DEVELOPMENT OF SKIN CANCER.

form of a topical cream.¹³ The liposome utilized in Dimericine is a fat bubble called a T4N5 liposome made from lipid lecithin, from the egg. It is thought to act via two mechanisms.

Immediately, T4 endonuclease V removes DNA dimers, primarily cyclobutane pyrimidine type. In the long-term, it may restore p53 gene function and exert a lasting chemopreventative effect. Dimericine has been studied as a topically applied cream to decrease the development of skin cancer in patients with xeroderma pigmentosum and renal transplant patients on immunosuppressive therapy. Dimericine received orphan drug designation for this indication in 1989.

In patients with xeroderma pigmentosum there is a rare genetic defect in UV radiation-induced DNA repair mechanisms characterized by severe sensitivity to all sources of UV radiation, especially sunlight. Several studies have been done using Dimericine to prevent skin cancer in xeroderma pigmentosum patients.

STUDIES ON DIMERICINE

One study found the Dimericine lotion reduced the incidence of basal cell carcinomas by 30% and of actinic keratoses by 68%.¹⁴

In another study, *in vivo* testing involving T4N5 liposome lotion has yielded intriguing results. In a test conducted with 12 xeroderma pigmentosum (XP) patients and 15 patients without this condition who had a history of skin cancer, researchers applied the cream at var-

ious intervals after controlled UV exposure. Biopsies conducted 6 hours after UV exposure revealed that patients with XP had achieved approximately 15% fewer CPDs (improved DNA repair) while patients with a history of skin cancer achieved less than 10% fewer CPDs.¹⁵

Phase I and II trials of Dimericine for prevention of skin cancer in xeroderma pigmentosum patients were completed. Dimericine, however, is not commercially available. The company states, "The XP Trial was registered with the FDA as a Phase III trial because it had a clinical endpoint: reduction of actinic keratoses and skin cancer and that its application is open. The FDA has undergone reorganization twice in the last 3 years and our application has been moved. We are discussing the number of XP patients required for market approval."

New studies of Dimericine are ongoing. Craig A. Elmets, M.D., Chair of the University of Alabama at Birmingham's Department of Dermatology and Senior Scientist at the UAB Comprehensive Cancer Center is leading a 3-year, multicenter, Phase II, randomized, double-blind controlled study of T4N5 liposome lotion, or Dimericine, to determine its success in preventing recurrence of non-melanoma skin cancer in 100 renal transplant patients. Enrollment is ongoing.

It would seem that the most useful role for Dimericine would be its inclusion in sun-block. Since it has no reported side effects, Dimericine is promising. Whether it will change clinical outcomes will become clearer as Phase III trials are completed.

PROMISING FUTURE

We now know that anti-oxidants and anti-inflammatory adjuvants are not the only ways to enhance the health of patients and retard the development of

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