Optigenex, Inc.

THE EFFICACY AND SAFETY OF AC-11®

Summary

AC-11: Description of the Product

AC-11 is an aqueous, hot water extract of the bark of the plant, *Uncaria tomentosa*. It is prepared by a process wherein the pulverized bark is heated to the sub-boiling point, centrifuged, and dialyzed. AC-11 contains less than 0.05% pentacyclic oxindole alkaloids and is standardized to carboxy alkyl ester content.

Data in Humans

Oral AC-11, when administered at doses from 250 to 400 mg/day for up to 8 weeks in human volunteers, has been shown to decrease DNA damage (8-hydroxyguanine DNA adducts and single-strand DNA breaks) and to increase DNA repair capacity (single-strand DNA breaks in leukocytes 30 minutes after DNA damage induced by hydrogen peroxide). Immune enhancement was observed in response to pneumococcal vaccine in volunteers given 700 mg/day of oral AC-11, but the effect of oral AC-11 (250 to 700 mg/day) on white blood cells was inconsistent. One study noted a significant reduction in pain (pre- vs. post-AC-11), while another reported a significant weight loss (also a pre- vs. post-AC-11 comparison). When applied topically in a 0.5% concentration, AC-11 was found to significantly reduce erythema following sun exposure and to produce a similar whitening of the skin as did 2% kojic acid following exposure to ultraviolet light.

AC-11 has been administered to an aggregate of 42 adult volunteers in 5 studies. In studies that reported adverse events (4 studies; 28 AC-11-treated volunteers), doses ranged from 250 to 700 mg/day and the duration of therapy ranged from 4 weeks to 60 days. No side effects were reported and only a few changes were noted in clinical chemistry and hematology profiles. A slight decrease in sodium was reported in two separate studies; however, in neither case did the decreases result in an abnormally low mean value. Mean serum iron concentration was reported to increase substantially (38.1%) in one study. The reported

mean increase in iron, however, was not abnormal when compared to published normal values.

In Vitro and Animal Data

AC-11, when administered orally to rats in 40 or 80 mg/kg/day doses, has been shown to significantly decrease oxidative DNA damage (single- and double-strand DNA breaks) induced in white blood cells by exposure to radiation or doxorubicin. *In vitro* studies have demonstrated significant decreases in photochemical DNA damage (cyclobutyl pyrimidine dimers) when AC-11 was applied topically (0.5%, 1.5%, and 3.0% solutions) to human living skin equivalents exposed to ultraviolet radiation.

AC-11 was effective in reducing the progression to malignant tumors in hairless mice repeatedly exposed to an escalating dose of UV-B radiation.

In vitro experiments demonstrated that AC-11 inhibits NF-κB in a dose-dependent manner over a broad range of concentrations (0.156 to 1.25 mg/ml) without inhibiting the degradation of IκBα.

Administration of oral AC-11 to rats and mice resulted in significant increases in lymphocyte and leukocyte proliferation. Following a leukopenia-inducing doxorubicin regimen, a significant shortening of the time until recovery from leukopenia was observed in rats that received 40 or 80 mg/kg/day of AC-11. At the 80 mg/kg/day dose, significant improvements were noted in both the neutrophil- and lymphocyte-rich fractions of white blood cells. By comparison, granulocyte colony stimulating factor given under the same circumstances resulted in a significant improvement only in the neutrophil-rich fraction.

When AC-11 was exposed to cultures of human leukemia and lymphoma cells, growth was inhibited without a significant increase in necrosis. At AC-11 concentrations up to 0.4 mg/ml, a delayed apoptosis was induced in leukemia and lymphoma cells without inducing necrosis. At a higher concentration (1.0 mg/ml) apoptosis was inhibited in human lymphoma cells with only modest necrosis observed.

Oral AC-11, administered to rats in doses of 40, 80, or 160 mg/kg/day for up to 8 weeks did not result in any significant changes in food consumption or weight gain.

Single oral doses of up to 8 g/kg did not result in deaths or signs of toxicity. Rats given 5 to 160 mg/kg of oral AC-11 showed no significant differences in liver, kidney, and spleen weights. One study did note that rats that were administered 80 mg/kg of AC-11 plus doxorubicin had a significantly increased mean heart weight coefficient (4.4%) when compared to animals that only received doxorubicin (0.386% ± 0.034 vs. 0.369% ± 0.022). However, there was no significant difference in mean heart weight when AC-11 plus doxorubicin treated animals were compared to untreated controls. Histopathological examination of tissues obtained from rats treated with 5 to 160 mg/kg/day of AC-11 for up to 8 weeks did not reveal an increase in pathological changes.

AC-11 Physical and Chemical Properties

AC-11

Description (Anon, 2002; Pero, 2000)

AC-11 is an aqueous, hot water extract of the bark of the plant, *Uncaria tomentosa*. It is a fine, beige to brown-orange, odorless hygroscopic powder which is soluble in water at concentrations greater than 400 mg/ml. It is slightly bitter to the taste. The AC-11 formulation is standardized to greater than 8% carboxy alkyl ester content.

AC-11 is currently formulated into vegetarian capsules, each containing the daily recommended dose of 350 mg. Previous formulations have included a 175 mg tablet (also marketed as AC-11) and a 350 mg capsule (marketed as C-MED-100).

Stability (Anon, 2002)

AC-11 content, as measured by the percentage of carboxy alkyl esters, has been shown to be stable over 4 years when stored away from heat, light, and moisture. The shelf life of AC-11 when stored in the original sealed container under dry conditions, between 15° and 40° C (59° — 104° F), is 3 years.

Method of Preparation (Pero, 2000)

The air dried bark or pulverized bark powder of *Uncaria tomentosa* is mixed with water and heated to the sub-boiling point for 20 to 24 hours. The dark brown, concentrated extract is adjusted for volume and filtered before centrifuging (3,000 x g) for 15 minutes at 40° C. The resultant particulate-free extract (equal to 150 grams of crude bark per 1,000 ml) is transferred in 50 ml aliquots into cellulose membrane dialysis tubing and dialyzed

against 1,000 ml of distilled water for 24 hours at 4° C. The light yellow fluid that diffuses out is concentrated by water vacuum evaporation at 50° C.

Chemical Composition

Alkaloid Content: AC-11 was analyzed using an HPLC technique. Six absorbance peaks were identified which corresponded to (in retention time order) Uncarine F, Speciophylline, Mitraphylline, Isomitraphylline, Pteropodine, and Isopteropodine (Sheng et al, 2000b), all of which are pentacyclic oxindole alkaloids. No tetracyclic oxindole alkaloids were detected. Indole alkaloids with molecular weights less than 10,000 daltons comprise less than 0.05% of the composition of AC-11 (Anon, 2002).

Non-Alkaloid Content (Anon, 2002): A variety of measurements of the non-alkaloid content of AC-11 has been conducted and is presented in Table 1.

Table 1. Non-Alkaloid Content of AC-11

Chemical	Amount Present
Chlorate Organics	0.01 ppm Maximum
Phosphate Organics	0.1 ppm Maximum
Carboxy Alkyl Esters	8% Minimum
Conjugated Tannins (with Molecular Weights > 10,000 Daltons)	0%
Conjugated Bioflavonoids (with Molecular Weights > 10,000 Daltons)	0%
Total Heavy Metal Content	10 ppm Maximum
Lead	1.4 ppm Maximum
Mercury	0.2 ppm Maximum

Inactive Ingredients in AC-11 Capsules (Anon, 2005)

Coloring Agents: There are no ingredients in AC-11 whose sole purpose is to provide color to the product.

Other Inactive Ingredients: AC-11 is contained in capsules free of animal proteins. Cellulose, magnesium stearate, and silica are also included in the formulation of AC-11.

AC-11 Efficacy

While the *in vitro* and *in vivo* effects of AC-11 are the subject of ongoing research, data collected and analyzed thus far indicate four major pharmacologic properties. They include favorable effects on DNA, NF-κB inhibition, immune enhancement, and tumor cell inhibition.

DNA Damage and DNA Repair Capacity

The Hazardous Environment In Which DNA Resides

Oxidants, DNA Damage, and Oxidative Stress: Free radicals are molecules capable of an independent existence, even if for only a brief period of time, that contain an unpaired electron (Fang et al, 2002; Young and Woodside, 2001). They tend to react readily with biomolecules such as proteins, amino acids, lipids, and DNA, resulting in cellular injury and death. The most important of the free radicals are those which are oxygen derivatives, reactive oxygen species.

Oxidative stress is a physiologic condition where the production of oxidants (such as free radicals and reactive oxygen species) exceeds the ability of the organism to neutralize them (Azzi et al, 2004; Turrens, 2003). The deleterious effects of oxidative stress are manifest as oxidative damage to cellular structures and functions. It has been estimated that the DNA in each human cell is chemically attacked by about 10,000 free radicals each day (Ames et al, 1993). They may come from exogenous sources such as sunlight, radiation, and environmental toxins; however, they also are derived from endogenous sources.

One source of endogenous free radical-containing reactive oxygen species occurs in humans as a normal consequence of cellular (mitochondrial) respiration and results in the production of hydroxyl radicals, hydrogen peroxide, and superoxide radicals (Finkel and Holbrook, 2000; McCord, 2000). The existence of superoxide dismutase enzymes in humans, as well as in all aerobic organisms, which functions exclusively to scavenge

reactive oxygen species in the form of superoxide anions (Finkel and Holbrook, 2000), is indicative of the dangers of unrestrained reactive oxygen species. Well over a dozen human gene variants appear to be directly involved in producing antioxidant enzymes that protect the body from oxidative stress (Fosberg et al, 2001).

The Effects of Oxidative Stress on Cells and on DNA: When cells are exposed to reactive oxygen species, damage can be observed in lipids, proteins, and DNA, including mitochondrial DNA (Mehlhorn, 2003; Esposito et al, 1999; Melov et al, 1999). On a cellular level, oxidative stress causes DNA-protein cross-linking, damage to the deoxyribose-phosphate component, and alterations in purine and pyrimidine bases (Dizdaroglu, 2002). 8-hydroxyguanine and thymine glycol are examples of oxidative changes to guanine and thymine, respectively (Figures 1 and 2; Bohr, 2002; Cheng et al, 1992). The concentrations of both compounds have been shown to increase in cells exposed to sources of oxidative stress, including ultraviolet light, ionizing radiation, and chemical mutagens that generate free radicals.

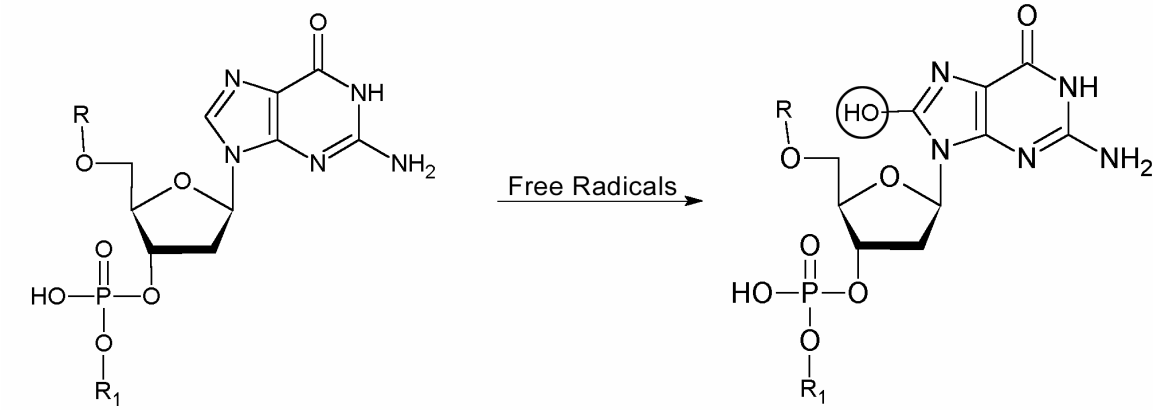

Figure 1. The oxidation of a guanine-containing nucleotide segment to 8-hydroxyguanine (adapted from Ichihashi et al, 2003). R and R_1 are adjacent nucleotides in the DNA chain. The alteration in the guanine base is shown circled.

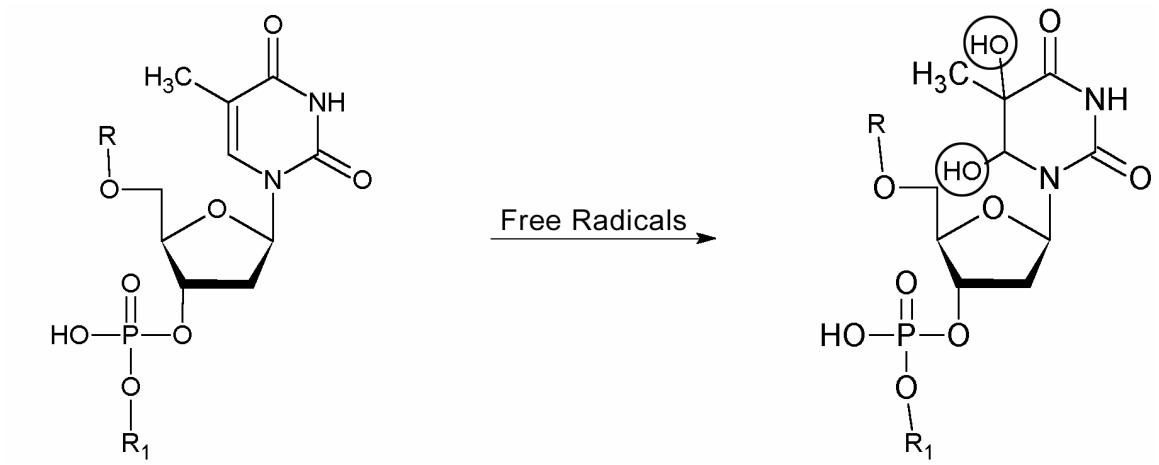

Figure 2. The oxidation of a thymine-containing nucleotide segment to thymine glycol (adapted from Ichihashi et al, 2003). R and R_1 are adjacent nucleotides in the DNA chain. The alterations in the thymine base are shown circled.

Over 100 different types of DNA lesions have been attributed to reactive oxygen species and these lesions are capable of disrupting vital cellular processes (Hoeijmakers, 2001). Damage to mitochondrial DNA, which occurs more readily than does damage to nuclear DNA (Finkel and Holbrook, 2000), causes impaired mitochondrial functionality, which could initiate a cyclic process that would yield additional increases in reactive oxygen species and still more DNA damage (Finkel and Holbrook, 2000). Further, it has been demonstrated that damage to mitochondrial DNA increases with age (Armbrecht, 2001). This may account for the fact that as we age, mitochondrial efficiency diminishes, resulting in decreased production of high energy compounds and increased production of free radicals.

Photochemical DNA Damage: Under strong sunlight, each exposed skin cell develops about 40,000 damaged DNA sites every hour (Ura and Hayes, 2002). While sunlight can produce potent oxidants (including singlet oxygen; Davies and Truscott, 2001), most of the genetic damage which results is distinctly different from that which occurs when DNA is exposed to free radicals. When ultraviolet light strikes DNA, adjacent pyrimidine bases (thymine and cytosine) bond together, forming dimers (Figure 3; Black et al, 1997). A series of cyclobutyl pyrimidine dimers are formed including thymine-thymine dimers (TT-dimers), thymine-cytosine dimers (TC-dimers),

cytosine-thymine dimers (CT-dimers), and cytosine-cytosine dimers (CC-dimers; Douki and Cadet, 2001). The formation of TT-dimers predominates. Additionally, pyrimidine (6-4) pyrimidone dimers (6-4 photoproducts), in which a bond forms linking positions 6 and 4 of adjacent pyrimidines and Dewer valence isomers (6-4 photoproducts with altered ring structures), are also produced.

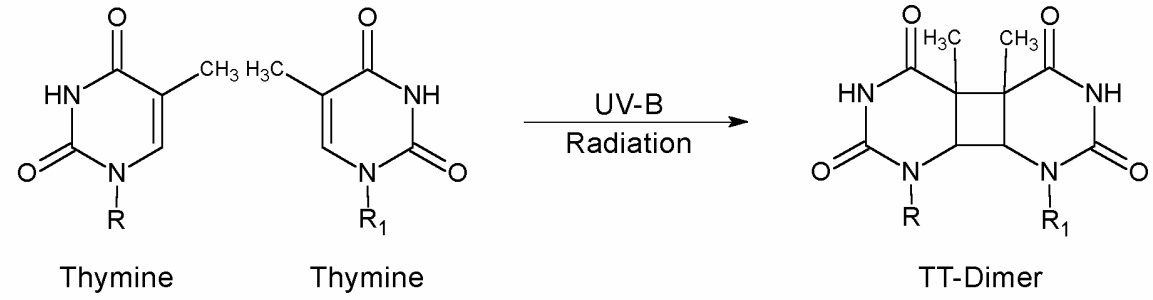

Figure 3. The formation of a cyclobutyl pyrimidine dimer from the energy of ultraviolet B light (UV-B) striking, in the case depicted, adjacent thymines (adapted from Ichihashi et al, 2003). R and R_1 are adjacent deoxyribose—phosphate groups in the DNA chain.

Evidence Supporting the Role of DNA Damage in the Aging Process

Aging, the process by which healthy adults become frail, experience diminished physiologic reserves, and much more readily develop diseases and die (Miller, 1998), is thought to result from the accumulation of somatic damage (Hasty et al, 2003). Some investigators contend that oxidative stress is the most important cause of somatic damage, and thus, the ultimate cause of aging.

Oxidative Stress, DNA Damage, and Longevity: Studies in flies and worms indicate that elevated levels of free radical scavenging enzymes increased longevity and resistance to oxidative stress (Arking et al, 2000; Larsen, 1998; Trower, 2000). Data obtained in rodents revealed that calorie restriction, which has been shown to almost universally prolong life span (Lee et al, 1999; Merry, 1999; Mobbs et al, 2001), reduced reactive oxygen species and the resultant oxidative damage (Merry, 2002).

As organisms age, increased levels of DNA with oxidative damage can be detected (Beckman and Ames, 1998). The

postulate that DNA damage, induced by oxidative stress (or other means for that matter), has a deleterious effect on longevity is supported by the fact that senescence can be induced by DNA damage (Campisi, 2003). Further, it had been established that replicative senescence occurs as a result of DNA losses (altered telomere structure) during normal DNA replication (Blackburn, 2000). Perhaps most importantly, the life span of several mammalian species was shown to correlate with the activity of a DNA repair process (Grube and Burkle, 1992).

DNA Damage and Aging in Humans: Studies in human fibroblasts revealed a prolonged life span in cells grown in low oxygen concentrations (Packer and Fuehr, 1977), while cells grown in high oxygen concentrations had a reduced life span (von Zglinicki et al, 1995). More importantly, the overproduction of an activated gene that triggers senescence-like state in fibroblasts resulted in increased oxidant levels and growth arrest (Lee et al, 1999; Serrano et al, 1997). This process was reversible by either reducing oxygen levels or by introducing an antioxidant.

The association between DNA damage and aging in humans is supported by a number of inherited diseases with specific gene mutations that manifest characteristics associated with accelerated aging (Hasty et al, 2003; Kyng and Bohr, 2005). For example, patients suffering from Cockayne's syndrome, a rare disease associated with defects in DNA repair, transcription, and apoptosis, exhibit thin hair, cachexia, retinal degeneration, hearing loss, neurodegeneration, and cataracts despite having a mean life span of about 20 years. Similarly, individuals with Werner syndrome, whose mean life span is less than 50 years, are predisposed to the early onset (in the second or third decade of life) of a variety of diseases associated with advanced age, including atherosclerosis, diabetes mellitus, osteoporosis, degenerative vascular disease, and cataracts. Individuals with Werner syndrome have been noted to have defects in DNA replication, recombination, repair, and transcription.

Mitochondrial DNA from mononuclear and polymorphonuclear leukocytes obtained from centenarians exhibited a strikingly high incidence of a mutation that altered the main replication origin of mitochondrial DNA (Zhang et

al, 2003). It has been speculated that this mutation alters the number of mitochondrial divisions per cell cycle, yielding younger mitochondria that are better able to cope with oxidative stress-induced damage.

In Vitro and Animal Studies Demonstrating The Beneficial Effects of AC-11 in Reducing DNA Damage and Increasing DNA Repair Capacity

DNA Repair Enhancement, Systemic: Two studies have been conducted in rats to assess the effect of AC-11 on the recovery from known sources of DNA damage (Table 2). In both cases, oral AC-11 therapy significantly increased DNA repair capacity.

Table 2. The effect of 40 mg/kg or 80 mg/kg of AC-11 on the recovery from sources of predictable DNA damage in Wistar-Furth rats (Sheng et al, 2000a; Sheng et al, 2000b).

Experiment Reference	n*	DNA Damage Induced By†	Duration of AC-11 Therapy	Outcome in AC-11-treated animals, compared with controls‡
A Sheng et al, 2000a	20	radiation	8 weeks	almost complete repair of SSBs substantially fewer DSBs at 40 mg/kg and significantly fewer at 80 mg/kg
B Sheng et al, 2000b	16	doxorubicin	5 days	significant decrease in SSBs at 80 mg/kg

* The number of rats studied.
† The agent employed to cause DNA damage.
‡ SSBs = Single Strand DNA Breaks; DSBs = Double Strand DNA Breaks; a significant change corresponds to $p < 0.05$.

Sheng et al, 2000a; Experiment A

Daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered to 20 female Wistar-Furth rats (10 rats per dose; random allocation to treatment groups) by gavage for 8 weeks (Sheng et al, 2000a). An additional 10 animals did not receive AC-11. Half the animals from each group were exposed to 12 Gy whole body radiation in a ^{137}Cs source and allowed 3 hours to repair *in vivo* before DNA damage was assessed. Five additional controls (untreated and unexposed to radiation) were also included in the experiment. Single strand DNA breaks and double strand DNA breaks were measured in splenic single cell suspensions by alkaline and neutral elution, respectively. Based on previous work using this model, after 3 hours about 50% repair of single DNA strand breaks would be anticipated in untreated controls (Sheng et al, 1998a). Both single and double strand DNA breaks were significantly greater in non-treated radiated animals compared to non-irradiated animals. AC-11-treated animals almost completely repaired single strand DNA breaks ($p < 0.05$) for both AC-11 doses compared to untreated, irradiated 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 when compared to untreated, irradiated animals.

Sheng et al, 2000b; Experiment B

Daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered orally, after dissolution in sterile tap water, to 8 Wistar-Furth rats (4 at each dose; random allocation to treatment groups) 24 hours after the last of three 2 mg/kg intraperitoneal doses of doxorubicin (48 hours separating each doxorubicin dose; Sheng et al, 2000b). Four animals were administered doxorubicin only and four received neither doxorubicin nor AC-11. Single strand DNA breaks were measured in splenic single cell suspensions by alkaline elution. Animals treated with 80 mg/kg of AC-11 had significantly ($p < 0.05$) reduced DNA damage.

DNA Repair Enhancement, Topical: Two in vitro studies demonstrated the effectiveness of AC-11 in protecting human living skin equivalents from UV-B-induce cyclobutyl pyrimidine dimers (TT-dimers). One of the studies also measured dimers immediately following UV-B exposure and found no significant change when compared to untreated controls. These data suggest that the beneficial effect

observed on TT-dimers was related to enhanced repair and not inhibition of dimer formation or UV-B screening.

Mammone et al, 2006; Experiment C

Excised portions of living skin equivalents (8 mm) were pre-treated with a 0.5% solution (5 mg per ml of sterile water) of AC-11 for 6 hours before being irradiated with 0, 50, 75, and 100 mJ/cm^2 type B ultraviolet light (UV-B; Table 3; Mammone et al, 2006). One group of living skin equivalents was fixed immediately, while another was re-treated with 0.5% topical AC-11. Samples were immunostained for thymine-thymine dimer (TT-dimer) formation and quantitated (counts of cells expressing TT-dimer staining) via light microscopy at 400x. TT-dimer levels immediately after UV-B exposure were not significantly different in living skin equivalents pre-treated with AC-11 when compared with cultures that were not exposed to topical AC-11 (Figure 4). By comparison, living skin equivalents re-treated with topical 0.5% AC-11 and evaluated 24 hours after UV-B exposure showed significant reductions in TT-dimer formation (Figure 5). Topical application of AC-11 to living skin equivalents exposed to 75 mJ/cm^2 had a significant ($p < 0.001$) 79% reduction in TT-dimer formation after 24 hours compared to a 54% reduction in TT-dimer formation in skin cultures not treated with AC-11. Living skin equivalents exposed to 100 mJ/cm^2 had a significant ($p << 0.001$) 73% reduction in TT-dimer formation in topical AC-11-treated cultures after 24 hours compared to an 11% reduction in TT-dimer formation in skin cultures not treated with AC-11.

Wachs, 2004; Experiment D

HaCaT keratinocyte cell cultures (human living skin equivalents) were pre-treated with a 0%, 0.5% (5 mg/ml), 1.5% (15 mg/ml), or a 3.0% (30 mg/ml) solution of AC-11 for 24 hours before being irradiated with 0 or 50 mJ/cm^2 type B ultraviolet light (UV-B; Table 3; Wachs, 2004). The UV-B light source was a Kodacel filtered FS40 sunlamp. Living skin equivalents that were treated only with vehicle (sterile water; 0% AC-11) were fixed immediately for assay while the other samples were incubated for 24 hours, fixed, and stained with antibodies specific to cyclobutyl pyrimidine dimers in DNA. Immunobinding was visualized by fluorescent microscopy and cyclobutyl pyrimidine dimers were measured using a semi-quantitative scoring system (CDP score). Living skin equivalents treated only with vehicle had a CDP score of 3.55 ± 0.64. Pre-treatment with a 0.5%, 1.5%, or 3.0% solution of AC-11 resulted in 21% (3.55 ±

0.64 vs. 2.81 ± 0.92), 25% (3.55 ± 0.64 vs. 2.66 ± 0.82), and 16% (3.55 ± 0.64 vs. 2.98 ± 0.60) decreases in CDP

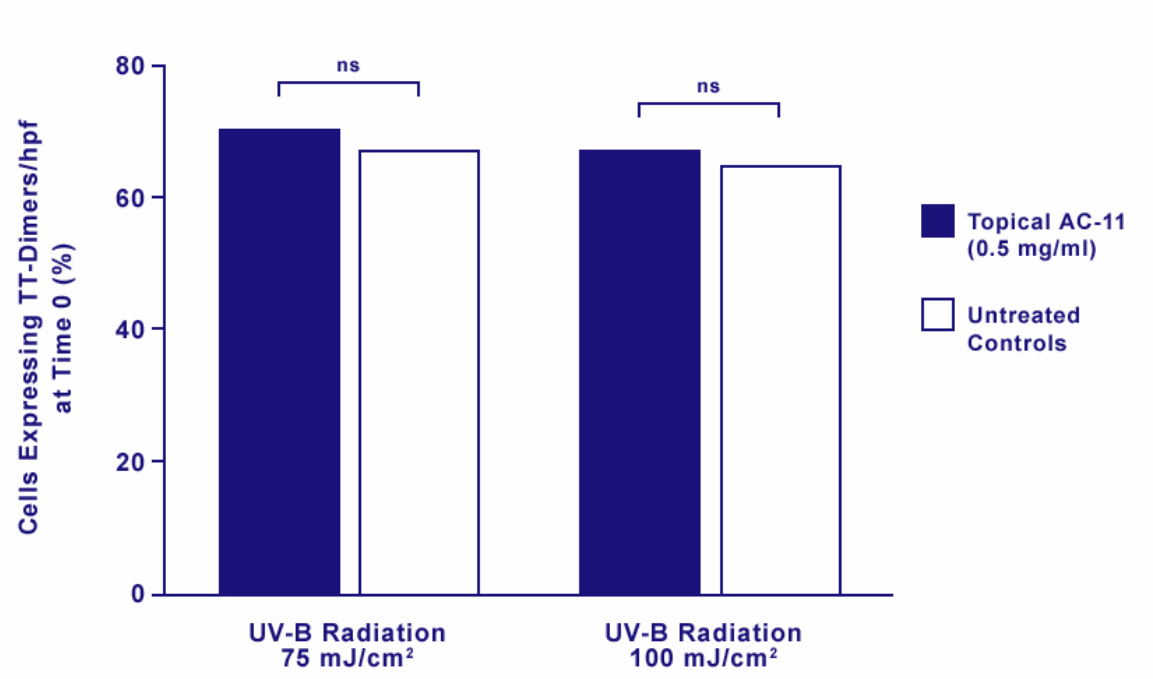

Figure 4. Percent of cells expressing TT-dimers per high powered field (hpf) immediately following UV-B exposure (75 or 100 mJ/cm^2). Cell cultures that were pre-treated with 0.5% AC-11 for 6 hours prior to UV-B exposure did not have significantly different dimer counts than untreated controls, suggesting that AC-11 neither prevented dimer formation nor blocked UV-B radiation (Mammone et al, 2006).

scores, respectively (Figure 6). In each case, pretreatment with topical AC-11 significantly ($p < 0.001$ in each case) decreased CDP score when compared to living skin equivalents treated only with vehicle.

Table 3. The effect of topical AC-11 on UV-B* induced cyclobutyl pyrimidine dimers in human living skin equivalents (LSEs; Mammone et al, 2006; Wachs, 2004).

Experiment Reference	UV-B Dose (mJ/cm^2)	AC-11 Concentration† (mg/ml)	AC-11 Dosing Schedule	Outcome
C Mammone et al, 2006	50, 75, and 100	5 (0.5%)	6 hour pre-treatment; UV-B exposure; then re-treatment	Significant ($p < 0.001$) reduction in TT-dimer‡ formation compared with controls (79% vs. 54%) in LSEs exposed to 75 mJ/cm^2 UV-B Significant ($p << 0.001$) reduction in TT-dimer‡ formation compared with controls (73% vs. 11%) in LSEs exposed to 100 mJ/cm^2 UV-B
D Wachs, 2004	50	5 (0.5%), 15 (1.5%), and 30 (3.0%)	24 hour pre-treatment; then UV-B exposure	Significant ($p < 0.001$ in all cases) 21%, 25%, and 16% reductions in CDP score§ were observed in LSEs treated with 0.5%, 1.5%, and 3.0% solutions of AC-11, respectively

*UV-B = Ultraviolet radiation, type B (280-320 nm).
†AC-11 was prepared as a solution in sterile water.
‡TT-dimers are a type of cyclobutyl pyrimidine dimer in which two adjacent thymines bond (see Figure 3)
§CPD Score = A semi-quantitative scoring system used to measure cyclobutyl pyrimidine dimers.

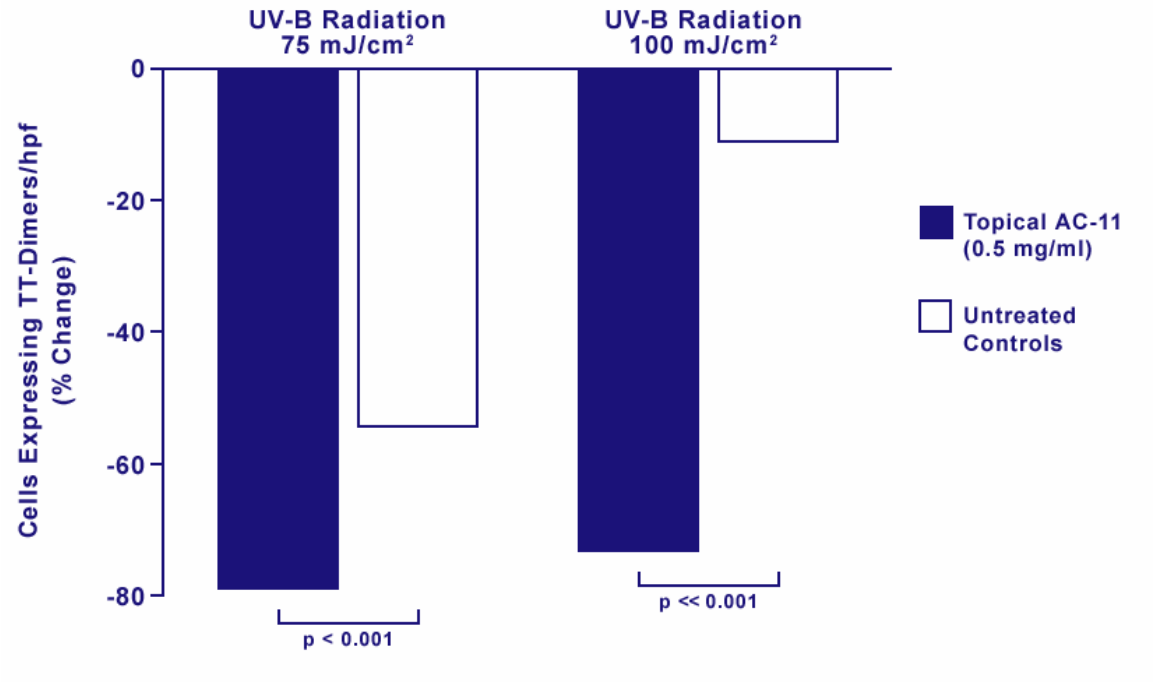

Figure 5. Percent of cells expressing TT-dimers per high powered field (hpf) 24 hours after UV-B exposure (75 or 100 mj/cm^2; Mammone et al, 2006).

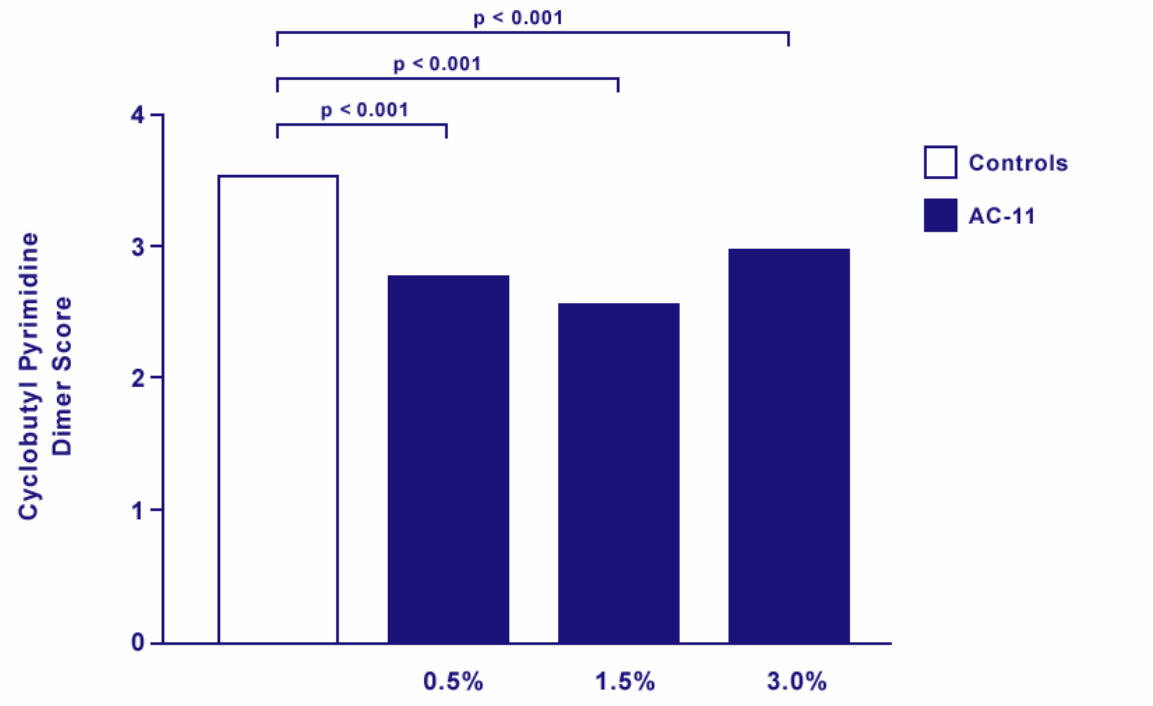

Figure 6. Significant reductions in cyclobutyl pyrimidine dimer scores in human living skin equivalents treated with 0.5%, 1.5%, or 3.0% AC-11, when compared to controls, 24 hours following UV-B exposure (50 mj/cm^2; Wachs et al, 2004).

Inhibition of Tumor Progression in Hairless Mice Repeatedly Exposed to Solar-Simulating Radiation

The Progression from DNA Damage to Cancer: DNA damage is commonplace. Oxidation alone is thought to result in thousands of damage events per cell each day (Ames et al, 1993; Helbock et al, 1998). Mutations (alterations in DNA which are passed to subsequent generations of cells), however, are remarkably infrequent. Normal cells have approximately 2×10^{-7} mutations per gene per cell division (Loeb, 2001). Boland and Goel (2005) pointed out, using the colon (a tissue comprised of an enormous mass of cells that are rapidly renewed and frequently exposed to mutagens) as an example, that if only a single mutation was sufficient to produce cancer, it would become an almost inevitable consequence of human existence. Thus, it does not appear that a single mutation is sufficient to produce a cancer. In the case of colorectal cancer, a theoretical minimum of 7 independent genetic events are necessary for the development of a malignancy (Kinzler and Vogelstein, 1996). However, a comparison of pre-malignant polyps and carcinoma cells found the average number of mutations per cancer cell to be about 10,000 (Stoler et al, 1999).

The mechanism whereby a series of mutations results in a malignancy is unclear. It appears likely that mutations which favor cell proliferation have a replicative advantage and that the progression to a malignancy occurs, in most situations, in a stepwise fashion (Loeb, 2001; Sieber et al, 2005; Boland and Goel,2005). However, a comparison of the rarity of mutations observed in normal cells with the large number of mutations detected in cancers suggests that cancers harbor mutations in the genes responsible for maintaining the genome (Loeb, 2001). Mutations in the genes responsible for genetic stability might result in an increased frequency of mutations, which, if they occurred in other areas critical for genomic stability could result in a cascade of mutations and the development of cancer. Critical mutations that could cause or contribute to genomic instability include those that occur in genes responsible for DNA synthesis and repair, cell cycle arrest, nuclease activation, and chromosome partitioning during division (Stoler et al, 1999).

Sunlight, the p53 *Gene, and Skin Cancer:* UV radiation is one of the few environmental agents that has been causally associated with cancer (Loeb, 2001). DNA repair is not perfect. The induction of large numbers of dimers and 6-4 photoproducts in the DNA of epidermal cells following exposure to sunlight, will, in some cases, result in mutations. Animal data suggest that a mutation in the critical *p53* gene (which is involved in DNA repair, cell cycle arrest, and apoptosis), may fail to abort the development of the pre-cancerous lesion, actinic keratosis (Ziegler et al, 1994). In normal cells, functional *p53* genes favor apoptosis of keratinocytes (sunburn) following overexposure to UV light. However, in actinic keratosis, subsequent exposures to UV radiation increase the likelihood that additional mutations and cancer (squamous cell carcinoma) will occur.

Skh-1 hairless mice represent a valuable model in skin cancer research because when repeatedly exposed to solar-simulating radiation (290 nm – 400 nm) they predictably develop squamous cell carcinoma (Willis et al, 1981). This provided an effective tool to assess whether reduced dimer burden, secondary to AC-11-induced repair, could mitigate the development of tumors.

Testing Whether a Reduced Dimer Burden Can Slow the Progression to Skin Cancer: Fifty Skh-1 hairless mice were repeatedly exposed to solar-simulating radiation using a 1,600 watt xenon arc lamp fitted with filters as a radiation source (Anon, 2006). Thirty mice were treated with AC-11 in 3 concentrations (0.5%, 1.5%, and 3.0%). AC-11 was formulated in a non-irritating, dye-free, perfume-free, and fragrance-free vanishing cream vehicle. Ten mice were treated with vehicle only and 10 were untreated. AC-11 or the vehicle only were applied daily to the backs of each mouse for the duration of the study.

Mice were initially exposed to 0.9 **x** the minimal erythemal dose 5 times a week for 2 weeks. The dose was increased by 20% and repeated 5 times a week during the next 2-week period. The 20% increase in radiation dose and the exposure schedule was continued for additional 2-week intervals. A total dose of 738 J/cm^2 of solar-simulating radiation was administered.

Tumor progression was assessed according to the following scale with the assigned score for each response noted in parentheses: mild erythema (0); intense macular erythema (0); light scaling accompanying erythema (1); firm scaling, palpable keratosis (2); raised palpable plaque, corresponding to early malignant development (3); and extensive tumor development (4). Mild erythema and intense macular erythema are not considered precancerous and were thus assigned a tumor progression score of zero.

Of the 50 mice exposed to solar simulating radiation, 5 were biopsied (one from each of the different AC-11 concentrations used, one from the vehicle-only group, and one from the untreated controls), for histological examination and not included in the assessment of clinical response. Other animals were not evaluable for reasons not related to the study or the test material. In total, 21 AC-11-treated and 14 vehicle-only controls or untreated controls were evaluable. Controls suffered more damage than did AC-11-treated mice (Table 4). The percent of animals that had a more severe clinical response was skewed to the animals who did not receive topical AC-11. For example, most of the AC-11-treated animals (52.4%) were found to have the least severe response observed (light scaling accompanying erythema). By comparison, only 14.3% of controls were found to have the same dermal manifestations following the same UV dose. More importantly, the percent of animals with the most severe clinical response (raised palpable plaques corresponding to early malignant development) occurred substantially more frequently in controls than in AC-11-treated animals (21.4% vs. 4.8%).

Table 4. Response to solar-simulating radiation at day 77 in hairless mice treated with or without topical AC-11. The total cumulative dose was 738 J/cm^2 (Anon, 2006).

	Controls* (n = 14)		AC-11† (n = 21)	
Response Category	Count	Percent	Count	Percent
Mild Erythema	0	0.0	0	0.0
Intense Macular Erythema	0	0.0	0	0.0
Light Scaling Accompanying Erythema	2	14.3	11	52.4
Firm Scaling, Palpable Keratosis	9	64.3	9	42.9
Raised Palpable Plaque Corresponding to Early Malignant Development	3	21.4	1	4.8
Extensive Tumor Development	0	0.0	0	0.0

* Controls included 7 mice that were treated with vehicle only and 7 that were untreated.
† AC-11 was applied topically in concentrations of 0.5%, 1.5%, and 3.0% to 7, 6,and 8 evaluable animals, respectively.

A significant ($p < 0.02$) difference was noted in the mean tumor progression scores when controls (2.07 ± 0.62) were compared to AC-11-treated animals (1.52 ± 0.60; Figure 7). There was no difference in mean tumor progression scores when responses to the different concentrations of AC-11 were compared. There was no difference in mean tumor progression scores when controls treated with vehicle-only were compared to untreated controls, suggesting that the vehicle had no significant effect on tumor progression.

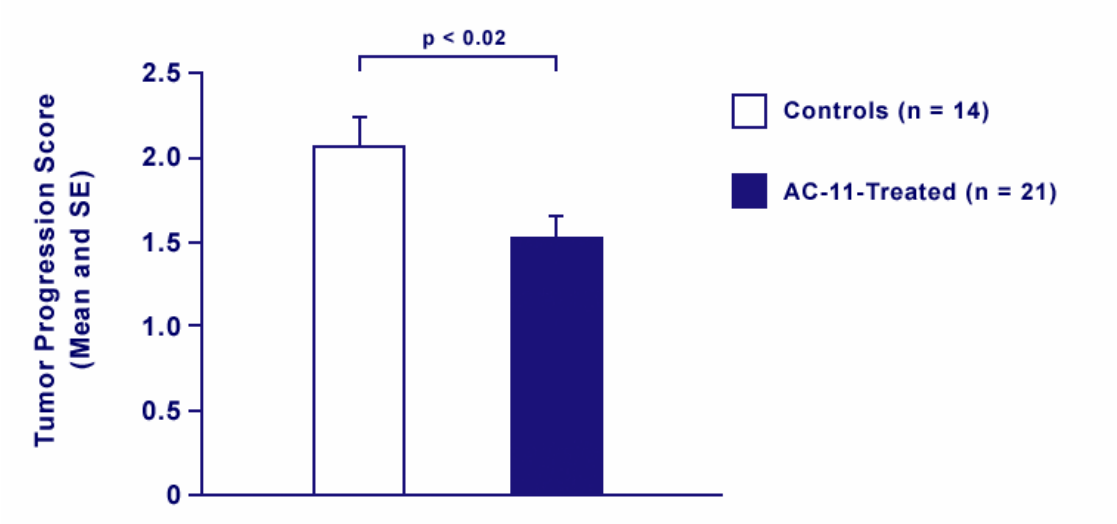

Figure 7. Mean (and standard error) tumor progression scores in hairless mice exposed to a cumulative dose of 738 J/cm^2 of solar-simulating radiation administered over 77 days (Anon, 2006). Control animals (n = 14) included untreated and vehicle-only-treated animals. Topical application of AC-11 (total n = 21) included animals treated with 0.5%, 1.5%, and 3.0% concentrations of AC-11. Tumor progression scores were assigned as follows: 4 = extensive tumor development; 3 = early malignancies (raised palpable plaques); 2 = firm scaling, palpable keratosis; 1 = light scaling with erythema.

Histological findings were consistent with the development of squamous cell carcinoma. Although it tended to be obscured by extensive hyperplasia, signs of collagen damage in irradiated areas and changes in dermal architecture were evident.

The Beneficial Effects of AC-11 on DNA in Humans

Decreased DNA Damage: Three studies have assessed the effects of AC-11 on DNA damage in humans (Table 5; Pero et al, 2002; Pero et al, 2005; Sheng et al, 2001). AC-11 was administered in oral doses of 250 to 400 mg per day for 4 to 8 weeks to small groups of healthy and apparently healthy volunteers, as well as volunteers being treated for chronic conditions. Two of the studies made comparisons to baseline assessments of DNA damage (Pero et al, 2002; Pero et al, 2005), while one included comparisons to an untreated control group (n = 4; Sheng et al, 2001). Two of the studies assessed 8-hydroxyguanine DNA adducts in blood (Pero et al, 2002; Pero et al, 2005) using the method of Park et al (1989), while one measured hydrogen peroxide-

induced DNA single strand breaks in blood (monocytes) by alkaline elution (Sheng et al, 2001).

All 3 studies reported a positive outcome with respect to the effect of AC-11 on DNA damage. Significant reductions in markers of DNA damage were reported in patients who received 350 mg of AC-11 per day for 4 and 8 weeks when compared with baseline and controls, respectively (Pero et al, 2002; Sheng et al, 2001). Most volunteers (9 of 14) in the study of Pero et al (2005) who received 400 mg of AC-11 per day had decreased 8-hydroxyguanine DNA adducts after 4 weeks; however, no inferential statistics were reported.

The study of Pero et al (2002) noted a significant ($p < 0.03$; $n = 5$) reduction in 8-hydroxyguanine adducts in peripheral lymphocytes after 4 weeks of AC-11 in combination with a vitamin, mineral, and antioxidant supplement (Figure 8). It must be noted, however, that the vitamin, mineral, and antioxidant supplement alone also resulted in a significant ($p < 0.01$; $n = 6$) decrease in 8-hydroxyguanine adducts after 4 weeks of therapy. The beneficial effect on DNA damage, however, persisted in AC-11 plus vitamin, mineral, and antioxidant supplement-treated volunteers ($p < 0.05$; $n = 4$) two weeks after therapy was discontinued, while no persistence of this effect was observed in the volunteers who received the vitamin, mineral, and antioxidant supplement without AC-11.

Table 5. The Effect of Oral AC-11 on DNA Damage in Human Volunteers.

Reference	Population	n[1]	Dose and Duration	Outcome
Pero et al, 2002	healthy volunteers between 35 and 55 years of age	5	350 mg/day for 4 weeks[2]	significant ($p < 0.05$) decrease in 8-hydroxyguanine DNA adducts compared to baseline which persisted (n = 4) two weeks after therapy was discontinued[5]
Pero et al, 2005	volunteers between 52 and 64 years of age; more than ¾ were being treated for various diseases[3]	14	400 mg/day for 4 weeks[4]	nine of the 14 participants had decreased 8-hydroxyguanine DNA adducts after AC-11 therapy only 1 participant had elevated DNA adducts prior to the intervention ($22/10^9$ neucleotide bases); it was reduced substantially ($2/10^9$ neucleotide bases) following AC-11 therapy
Sheng et al, 2001	apparently healthy volunteers	8	250 or 350 mg/day for 8 weeks	significant ($p < 0.05$) decrease in DNA single strand breaks[6] in volunteers who received 350 mg/day (n = 4) for 8 weeks[7]

[1]The number of AC-11-treated volunteers.
[2]A vitamin/mineral/antioxidant supplement was administered with AC-11.
[3]Including: cardiac disease, hypertension, diabetes, hysterectomy, thyroid disease, allergy, arthritis, and polio.
[4]In addition to AC-11, the supplement administered included nicotinamide, zinc, and KMA complex (mushroom extracts).
[5]Six participants who only received the vitamin/mineral/antioxidant supplement also had increased 8-hydroxyguanine DNA adducts after 4 weeks of therapy, compared to baseline ($p < 0.01$).
[6]The resistance to DNA damage was assessed using *in vivo* treated mononuclear leukocytes exposed to 100 µM of hydrogen peroxide and measuring DNA single strand breaks by alkaline elution.
[7]The number of DNA single strand breaks prior to completing 6 weeks of AC-11 therapy was not significantly different compared to control. The number of DNA single strand breaks was not significantly different in mononuclear leukocytes of volunteers treated with 250 mg of AC-11 per day at any time measured.

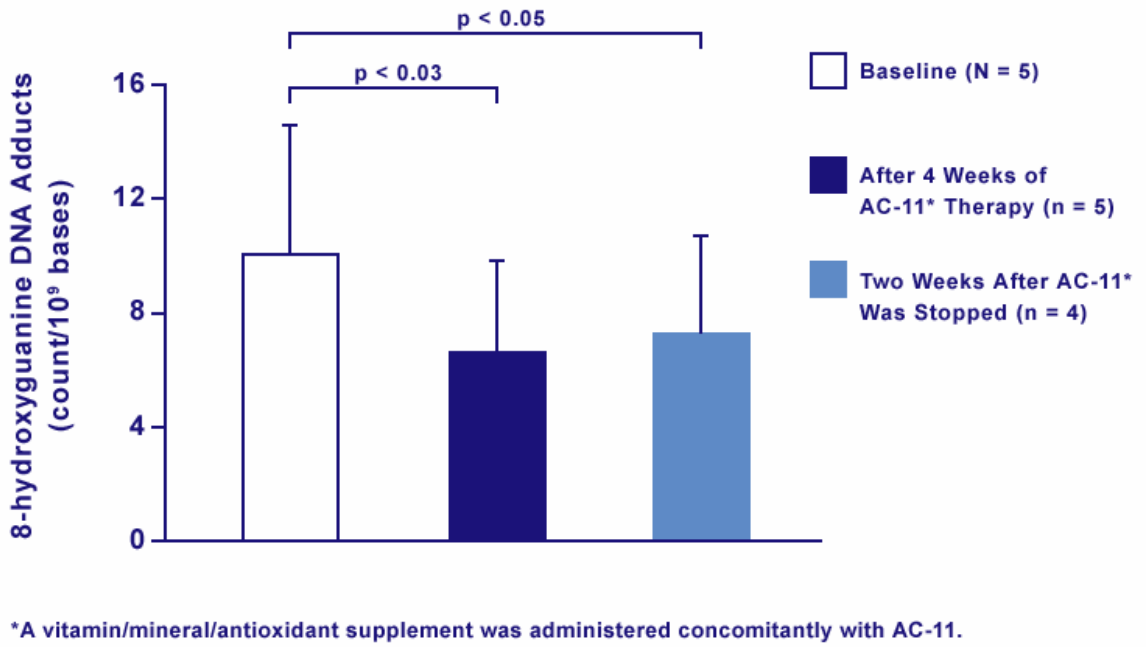

Figure 8. The frequency of oxidative DNA damage, as measured by 8-hydroxyguanine DNA adducts in lymphocytes, 4 weeks after oral administration of 350 mg of AC-11 and a vitamin, mineral, and antioxidant supplement daily (Pero et al, 2002). A significant reduction in oxidative DNA damage was noted to persist 2 weeks after AC-11 therapy was discontinued.

Sheng et al (2001), observed a significant ($p < 0.05$) decrease in DNA single-strand breaks in the monocytes of healthy volunteers who received 8 weeks of 350 mg/day of AC-11; however, no significant changes were noted in volunteers who received 250 mg/day.

Increased DNA Repair Capacity: Twelve healthy volunteers received oral AC-11 doses of 250 mg (n = 4) or 350 mg (n = 4) for 6 weeks and an additional 4 volunteers received no therapy (Sheng et al, 2001). DNA repair capacity was assessed by measuring DNA single-strand breaks in monocytes 30 minutes after *in vitro* exposure to a known DNA damaging agent (100 µM hydrogen peroxide). A significant reduction in mean DNA single-strand breaks compared to baseline was observed in volunteers who received 250 mg/day or 350 mg/day (Figure 9). No such effect was noted in volunteers who did not take AC-11.

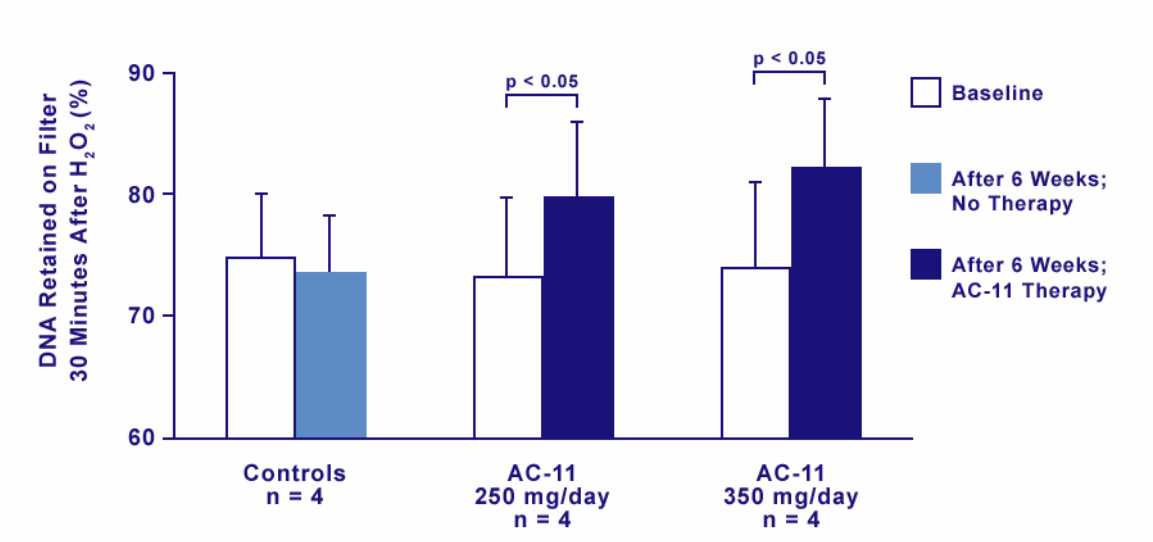

Figure 9. Significant improvement in DNA repair capacity in volunteers who received 250 mg/day or 350 mg/day of AC-11 for 6 weeks (Sheng et al, 2001). DNA single-strand breaks were measured by alkaline elution. An increase in percent DNA retained on the filter is indicative of decreased single-strand DNA breaks.

Mechanism of Action for the Beneficial Effect Observed on DNA

The pharmacologic mechanism for the beneficial effect that AC-11 exerts on DNA has not been investigated. It has been speculated that antioxidant properties may be responsible for a reduction in oxidative stress, thus improving DNA repair capacity (Sheng et al, 2000a). While other *Uncaria tomentosa* extracts have shown antioxidant properties (Desmarcheilier et al, 1997; Sandoval et al, 2000), AC-11's ability to scavenge free radicals or otherwise reduce the quantity or negative effects of reactive oxygen species has not been investigated.

Even if one were to assume that, like other *Uncaria tomentosa* extracts, AC-11 has inherent antioxidant properties, those properties alone would not account for the diverse beneficial effects that AC-11 has on DNA. For example, the positive effects obtained in studies that measured single- and double-strand DNA breaks, as well as oxidative nucleic acid products, are consistent with scavenging of free radicals (Beckman and Ames, 1998, Fang et al, 2002, Shao et al, 1999; Thompson and West, 2000). In these cases, it is unclear if the beneficial effects of AC-11 are due to a yet undefined pharmacologic mechanism, an antioxidant effect, or a combination of the two.

Following topical application, however, AC-11 has been shown to significantly enhance the repair of TT-dimers. Although their formation is associated with erythema, inflammation, reduced immune response, mutations, and skin cancer (Vink and Roza, 2001), the generation of TT-dimers is not an oxidative process. Dimerization occurs when UV light strikes thymine and cytosine, forming covalent bonds between adjacent pyrimidines, producing cyclobutyl pyrimidine dimers (including TT-dimers) and 6-4 photoproducts (Black et al, 1997). Since the formation of TT-dimers is a direct effect of ultraviolet radiation, antioxidants would not be expected to affect their formation or repair as has been demonstrated with AC-11. In fact, a study in hairless mice demonstrated that topical application of an antioxidant (olive oil), immediately after UV-B exposure, significantly ($p < 0.05$) reduced oxidative DNA damage (8-hydroxy-deoxyguanosine) while cyclobutyl pyrimidine dimers and 6-4 photoproducts were unchanged (Budiyanto et al, 2000).

DNA Associated Effects: Erythema and Sunburn

Evidence that Epidermal DNA Damage and Erythema Are Interrelated Phenomena: Chromophores are chemicals that absorb light and, as a consequence, may initiate a biologic response (Young, 1997). Human skin contains several classes of chemicals that absorb ultraviolet light at unsaturated bonds, including proteins, melanins, and DNA. The propensity for ultraviolet light to generate photochemical DNA damage, including pyrimidine dimers, is well established (Black et al, 1997; Ura and Hayes, 2002). Young et al (1998) compared photobiologic efficiencies (action spectrums) and found remarkable similarities for erythema and TT-dimers between 300 and 340 nm. These data suggest that DNA is a major chromophore for erythema and that photochemical skin damage and erythema are closely interrelated.

Although sunburn is grossly perceived as erythema, on a cellular level it is manifested as keratinocytes undergoing apoptosis (Van Laetham et al, 2005). This apparent protective effect is triggered by UV-induced sun damage and is likely mediated by the *p53* gene (Ziegler et al, 1994). A mutation in *p53* could favor the growth of a group of cells defective for sunburn cell production which, following subsequent sun exposures, fail to halt the progression to actinic keratosis.

Measurement of Sunburn Cells in Living Skin Equivalents: Excised portions of living skin equivalents (8 mm) were pre-treated with a 0.5% solution (5 mg per ml of sterile water) of AC-11 for 6 hours before being irradiated with 0, 50, 75, and 100 mJ/cm^2 type B ultraviolet light (UV-B; Mammone et al, 2006). One group of living skin equivalents was fixed immediately, while another was re-treated with 0.5% topical AC-11 for an additional 24 hours. Samples were fixed in formalin and stored at -4° C prior to staining and histopathological examination for necrotic cells (sunburn cells) under light microscopy at 400x. There were no necrotic cells detected (with or without topical AC-11 treatment) after exposure to 0, 50, or 75 mJ/cm^2 UV-B. However, living skin equivalents treated with topical AC-11, and exposed to 100 mJ/cm^2 UV-B, had significantly ($p < 0.001$) fewer sunburn cells when compared to living skin equivalents not treated with topical AC-11 and exposed to 100 mJ/cm^2 UV-B.

Gross Assessment of Erythema Following Sun Exposure in Human Volunteers: Forty-two volunteers applied two different topical products prior to sun exposure (Scheinfeld and Wachs, 2005). One of the products was applied to the right side and the other to the left side of their bodies. The two products were 0.5% AC-11 plus an SPF 15 sunscreen and the SPF 15 sunscreen only. The products were marked with a code and were otherwise indistinguishable. The investigators assigned which product the volunteers applied to each side. The volunteers were unaware which product contained AC-11 and thus which side was treated with topical AC-11. After sun exposure, volunteers were asked to evaluate the response to the different topical formulations with respect to erythema, blistering, and pain.

The frequency of erythema in AC-11 plus sunscreen-treated sites (6 of 42), was significantly ($p < 0.0001$) reduced compared with sites that were treated with sunscreen only (33 of 42; Figure 10). A significant ($p < 0.0001$) reduction was also noted in the incidence of blistering (4 of 42 AC-11 plus sunscreen-treated sites and 33 of 42 sunscreen only-treated sites). Pain was reported by 2 volunteers on the sunscreen-only site and by none on the AC-11 plus sunscreen sites (not significant).

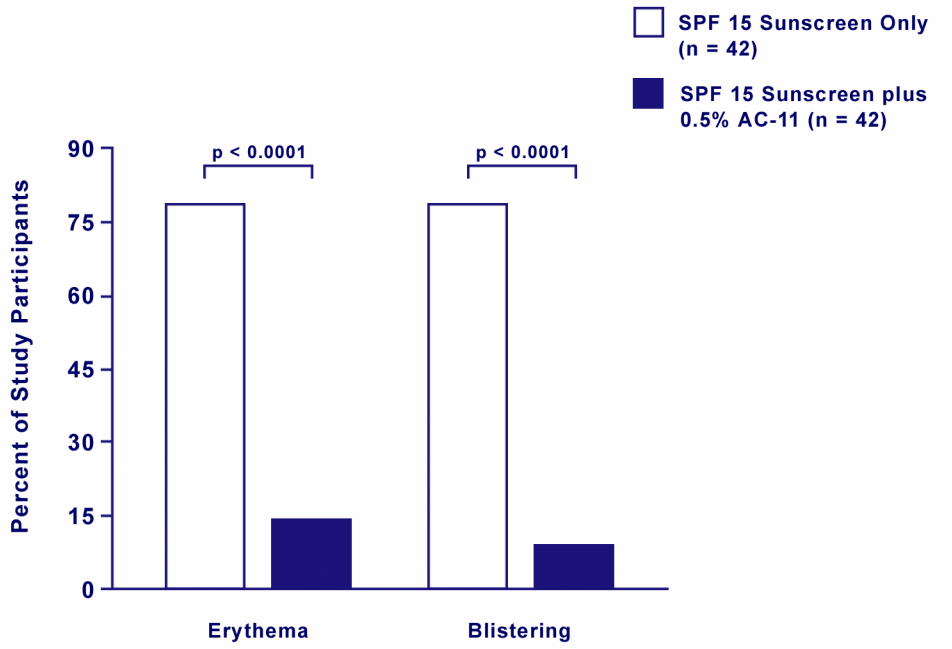

Figure 10. The percent of study participants who noted erythema and blistering on sites where sunscreen only and AC-11 plus sunscreen formulations were applied prior to sun exposure (Scheinfeld and Wachs, 2005).

NF-κB Inhibition

NF-κB, An Overview

The transcriptional factor NF-κB has been shown to influence a variety of biological functions, one of which is inflammation. NF-κB is mostly contained in the cytoplasm of cells in combination with the inhibitor protein, IκB (Baldwin, 1996). Once separated, NF-κB migrates into the nucleus where it activates genes encoding immunologically relevant proteins (Baeuerle and Henkel, 1994). The activation of NF-κB occurs under a variety of disparate conditions, including exposure to bacterial toxins, viruses and viral products, mitogens, UV light, γ-radiation, and oxidative stress (Baeuerle and Henkel, 1994). Most of the stimuli that result in NF-κB activation reflect exposure to pathologic conditions.

NF-κB has been associated with inflammatory diseases such as rheumatoid arthritis, asthma, and inflammatory bowel disease (Baldwin, 2001; Tak and Firestein, 2001). Additionally, it has been shown to be activated in the

synovia of arthritics and can be inhibited by anti-arthritic therapies, including aspirin, prednisone, and gold (Baldwin, 1996).

AC-11 and NF-κB Inhibition

Three *in vitro* experiments were conducted to assess the effect of AC-11 on NF-κB (Åkesson et al, 2003a; Table 6).

Table 6. The *in vitro* Effect of AC-11 on NF-κB (Åkesson et al, 2003a)

Experiment	Cell Type	AC-11 Concentration(s)*	Outcome
E	Mouse lymphocytes	0, 0.156, 0.312, 0.625, 1.25	Igκ† was inhibited
F	Human T-cells	0, 0.5, 1.0	NF-κB activity decreased
G	Mouse lymphocytes	1.0	No inhibition of LPS-induced‡ IκBα degradation

* Concentrations reported in mg/ml.
† NF-κB inhibition would be expected to decrease Igκ levels (Zhang and Ghosh, 2001; see below).
‡ LPS = lipopolysaccharide.

Åkesson et al, 2003a; Experiment E

70Z/3 mouse pre-B lymphocyte cells were pre-treated with 0, 0.156, 0.312, 0.625, and 1.25 mg/ml of AC-11 for 3 or 4 hours then stimulated with lipopolysaccharide (25 µg/ml) for 20 or 24 hours before staining with 7-amino-actinomycin D and Igκ antibodies. Igκ-expression was measured by flow cytometry. The investigators reported that Igκ positive cells were inhibited in a dose-dependent manner (Figure 11). Igκ activity decreased approximately 15% when cultures pre-treated with 0.156 mg/ml and 1.25 mg/ml of AC-11 were compared. NF-κB binds to an enhancer element in the B-cell specific Igκ gene (Zhang and Ghosh, 2001). Thus, inhibition of NF-κB would be reflected in Igκ levels.

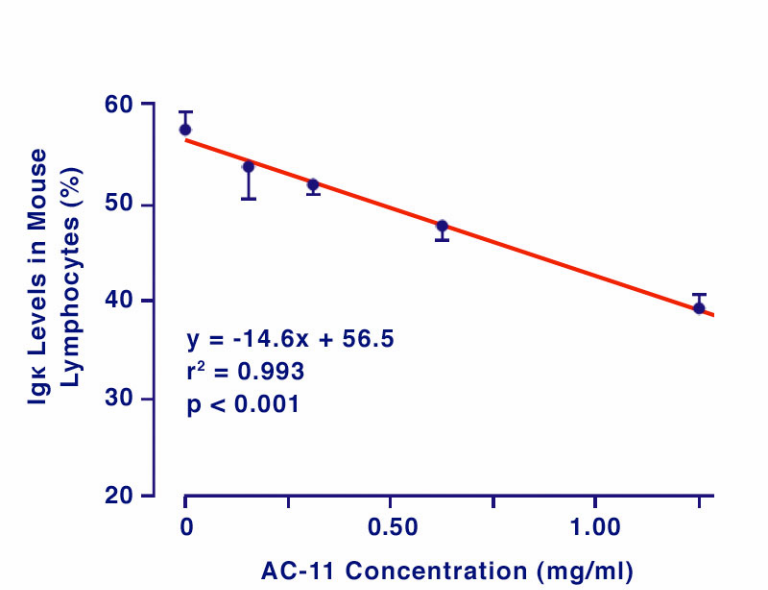

Figure 11. The linear, concentration-dependent effect of AC-11 on Igκ expression in mouse lymphocytes (adapted from Åkesson et al, 2003).

Åkesson et al, 2003a; Experiment F

Jurkat human acute T-cell leukemia cells, transfected with a construct carrying NF-κB activated luciferase reporter gene, were pre-treated with 0, 0.5, and 1.0 mg/ml of AC-11 for 2 hours and then stimulated with PMA and ionomycin for 6 hours before measuring luciferase activity. At the highest concentration tested, AC-11 was found to reduce NF-κB activity by almost 50% (Figure 12).

Åkesson et al, 2003a; Experiment G

70Z/3 mouse pre-B lymphocyte cells were pre-treated with either 1 mg/ml of AC-11 or pyrollidine dithiocarbamate (positive control) for 2 hours after which they were stimulated with lipopolysaccharide (25 µg/ml) for 30 minutes and for 3 hours before analysis of IκBα antibodies by Western blotting. AC-11 was found not to inhibit the lipopolysaccharide-induced degradation of IκBα.

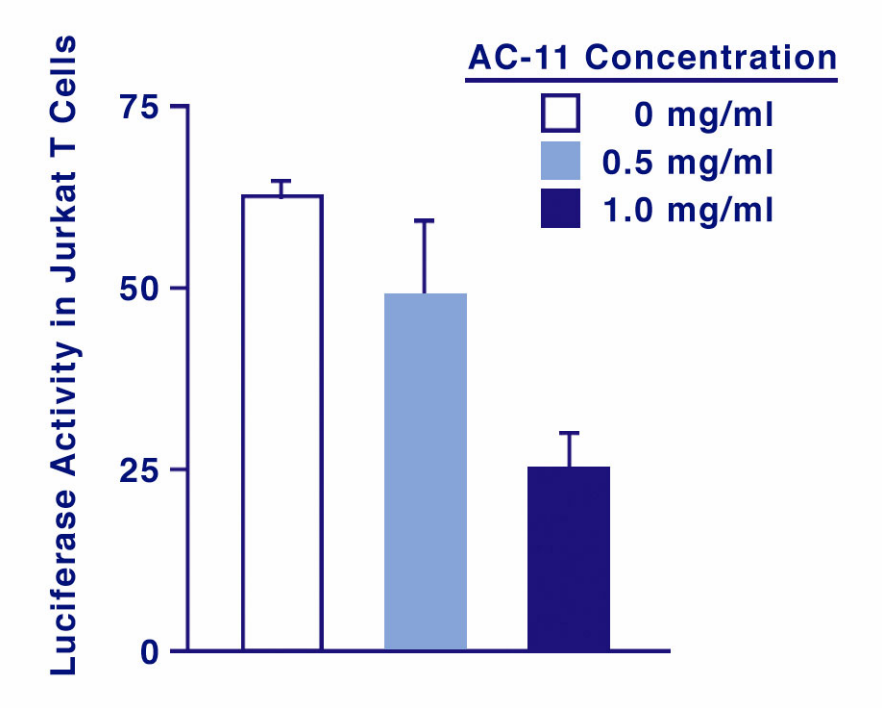

Figure 12. Luciferase activity in human Jurkat T cells transfected with an NF-κB reporter construct (adapted from Åkesson et al, 2003).

Immune Enhancement

Immune Enhancement in Animal Studies

Reversal of Leukopenia: Daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered orally to 22-24 Wistar-Furth rats (11-12 at each dose) 24 hours after the last of three 2 mg/kg intraperitoneal doses of doxorubicin (48 hours separating each doxorubicin dose; Sheng et al, 2000b). Two additional study groups (11 – 12 animals per group) were included in the experiment; in one group the animals received only doxorubicin, while the other included untreated controls. Blood samples were obtained from rat orbital vessels and analyzed for total white blood cell counts over a 3 week period. White blood cell counts were noted to predictably drop following the second doxorubicin dose and continued falling after the third dose. White blood cell counts would be expected to recover 3 weeks after the last doxorubicin dose. The recovery of white blood cell counts occurred significantly ($p < 0.05$) sooner in AC-11-treated animals, at day 10 and day 15, after 4 and 9 days of AC-11 therapy, respectively.

In the same report, using the same leukopenia-inducing dosing schedule of doxorubicin, the investigators compared the effect of subcutaneously administered granulocyte colony stimulating factor, 5 µg/kg and 10 µg/kg, with 40 mg/kg and 80 mg/kg oral AC-11. Also included were doxorubicin-treated only and untreated controls (10 or more Wistar-Furth rats per group). Blood was obtained and white blood cell counts were reported as lymphocyte rich fractions and neutrophil rich fractions. Granulocyte colony stimulating factor therapy resulted in significantly ($p < 0.05$) greater neutrophil rich fractions of white blood cells when compared to doxorubicin only at both doses. By comparison, the animals treated with 80 mg/kg had significantly ($p < 0.05$) greater neutrophil rich and lymphocyte rich fractions of white blood cells. No significant white blood cell changes were observed in the group that received 40 mg/kg of AC-11.

Lymphocyte Proliferation: Wistar-Furth rats were treated for 8 weeks with 0, 5, 10, 20, 40, and 80 mg/kg of oral AC-11 (at least 5 animals per group) after which splenic single cell suspensions were prepared and stimulated with phytohemagglutinin for 5 days (Sheng et al, 2000a). Statistically significant increases in the tendency of mitogenic response were observed in animals treated with 40 mg/kg ($p < 0.05$) and at 80 mg/kg ($p < 0.01$) compared with untreated controls.

Leukocyte (Splenic) Proliferation: Female C57BL/6 mice were fed AC-11 in drinking water at 0, 1, 2, and 4 mg/ml concentrations for 24 days (Åkesson et al, 2003b). A total of 18 mice were included in the experiment; 7 in the 0 mg/ml group, 2 in the 1 mg/ml group, 2 in the 2 mg/ml group, and 7 in the 4 mg/ml group. Mice fed drinking water containing 4 mg/ml of AC-11 had significantly ($p < 0.05$) greater mean spleen cell (leukocyte) count compared to control.

Immune Enhancement in Humans

Increased Antibody Titers: Twenty-three male Caucasian volunteers between 40 and 60 years of age, with no apparent acute diseases, were randomly assigned to receive either 700 mg of oral AC-11 (350 mg twice a day; n = 12) or no supplementation (n = 11) for 60 days (Lamm et al, 2001). On day 30 the volunteers were given 0.5 ml pneumococcal vaccine (Pneumovax), intramuscularly. Pneumococcal

antibody titers (estimates of IgG responses to pneumococcal serotypes 1, 3, 4, 6, 8, 9N, 12, 14, 18, 19F, 23F, 51, and 56) were determined on day 30 (prior to administration of the vaccine), on day 60, and on day 180. AC-11-treated volunteers had a statistically significant immune enhancement, both in percentage of antibody titers ($p < 0.01$) and in average antibody titers ($p < 0.05$), compared with controls after 1 month (60 days of AC-11 therapy). No significant differences in antibody titers were observed on day 180 (5 months after pneumococcal vaccine and 4 months after AC-11 therapy was discontinued).

Leukocytosis: AC-11 therapy in human volunteers has not produced consistent results on white blood cell counts (Table 7). Although one study noted a significant increase in total white blood cell counts in a small group of volunteers after 6 weeks of 350 mg/day of AC-11, other studies with larger populations, higher doses, and longer durations of therapy did not corroborate a white blood cell inducing effect.

AC-11 was administered to four apparently healthy adult male volunteers (32 to 58 years of age) at a dose of 350 mg once daily for 6 weeks (Sheng et al, 2000a). Patients were found not to have altered food intake patterns, lifestyles, or medications during AC-11 administration, and no new diseases were diagnosed. Total white blood cell counts were obtained before AC-11 therapy (3 measurements for each participant) and after AC-11 therapy (4 measurements for each participant; the last 4 weeks of data). The mean white blood cell count after AC-11 therapy (7.18 ± 0.50) was significantly ($p < 0.05$) greater than the mean white blood cell count after therapy (6.60 ± 0.35).

A total of 12 male Caucasian volunteers between 40 and 60 years of age, with no apparent acute diseases, received 700 mg of oral AC-11 (350 mg twice a day) for 60 days (Lamm et al, 2001). On day 30 the volunteers were given 0.5 ml pneumococcal vaccine, intramuscularly. Blood samples were obtained on day 0 (before AC-11 administration) and on day 60, and sent to a commercial laboratory for analysis of various hematologic parameters (Table 8). Mean total white blood cell count was noted to decrease following AC-11 therapy (before = $6.46 \pm 1.04 \times 10^3/mm^3$; after = $5.76 \pm 0.66 \times 10^3/mm^3$). This did not achieve statistical significance ($p > 0.05$). Only the change in polymorphonuclear neutrophils (before = 4084 ± 1121 mm^3; after = 3243 ± 836 mm^3) achieved statistical significance ($p < 0.05$).

Table 7. The effect of AC-11 on white blood cell counts in human volunteers.

Reference	n*	AC-11 Dose (mg/day)	Duration of Therapy	Outcome†
Sheng et al, 2000a	4	350	6 weeks	Increased WBCs ($p < 0.05$)
Lamm et al, 2001	12	700‡	60 days	Decreased WBCs (NS) Decreased polys ($p < 0.05$)
Sheng et al, 2001	12	controls (n = 4) 250 (n = 4) 350 (n = 4)	8 weeks	WBCs almost unchanged (NS) Decreased platelets ($p < 0.05$)

* The number of human volunteers studied.
† WBCs = mean total white blood cell count; polys = polymorphonuclear neutrophils; NS = not significant ($p \geq 0.05$); MCHC = mean corpuscular hemoglobin concentration. Comparisons made between before and after AC-11 therapy unless stated.
‡ Pneumococcal vaccine was administered on day 30.

Twelve apparently healthy volunteers (6 males and 6 females; between 28 and 54 years of age) were randomly assigned to receive either 0, 250, or 350 mg/day of AC-11 for 8 weeks (Sheng et al, 2001). Patients were found not to have altered food intake patterns, lifestyles, or medications during AC-11 administration and no new diseases were diagnosed. Hematologic parameters, including total white blood cell counts, were obtained before and after AC-11 therapy. There were no significant differences in mean total white blood cell count before and after AC-11 therapy in volunteers treated with 250 mg/day (before = $5.4 \pm 1.1 \times 10^3/mm^3$; after = $5.5 \pm 1.5 \times 10^3/mm^3$) or 350 mg/day (before = $5.7 \pm 0.4 \times 10^3/mm^3$; after = $5.7 \pm 0.8 \times 10^3/mm^3$).

Table 8. Hematology Values Obtained Before and After 60 Days of AC-11 Therapy in 12 Volunteers With No Apparent Acute Diseases (Lamm et al, 2001)

Hematology Test	Significant Change
Total White Blood Cells	No
Total Red Blood Cells	No
Hemoglobin	No
Hematocrit	No
Mean Corpuscular Volume	No
Mean Corpuscular Hemoglobin	No
Mean Corpuscular Hemoglobin Concentration	No
Red Blood Cell Distribution Width	No
Mean Platelet Volume	No
Total Platelet Count	No
Total Polymorphonuclear Neutrophils	Yes*
Percent Polymorphonuclear Neutrophils	No
Total and Percent Lymphocytes	No
Total and Percent Monocytes	No
Total and Percent Eosinophils	No
Total and Percent Basophils	No
Total and Percent CD3 Lymphocytes	No
Total and Percent T-Helper Lymphocytes	No
Total and Percent T-Suppressor Lymphocytes	No
T-Helper/T-Suppressor Ratio	No

*Significant ($p < 0.05$) difference in mean polymorphonuclear neutrophil count before AC-11 therapy (4084 ± 1121 mm^3) and after AC-11 therapy (3243 ± 836 mm^3).

Antitumor Activity

Cultures of human leukemic cell lines HL60 and K562, and the lymphoma cell line Raji were incubated for 72 hours after the addition of 0, 100, 200, 300, and 400 µg/ml of AC-11 (Sheng et al, 1998b). ^{3}H-Thymidine incorporation and colorimetric MTT assay techniques both demonstrated a dose-dependent inhibition of cell proliferation. AC-11 was a more potent inhibitor of HL60 and Raji cells than K562 cells.

HL60 human lymphoma cells, Raji human Burkitt's lymphoma cells, and Jurkat human acute T-cell leukemia cells were

cultured in the absence or presence of 0.25, 0.5 and 1.0 mg/ml of AC-11 and stained with 7-amino-actinomycin D (2 µg/ml) and Annexin V, and cell counts performed at various times over 4 days after the start of culture by flow cytometry (Åkesson et al, 2003a). A dose-dependent inhibition of the growth of human tumor cells was observed, Raji and HL60 to a greater extent than with Jurkat. However, there was no significant increase in the number of dead cells at any of the AC-11 concentrations, compared to control.

Cultures of human Jurkat leukemic cells and murine leukemic cells were seeded in 25 cm^2 flasks (100,000 cells/ml) and immediately exposed to 0, 0.125, 0.25, 0.5, and 1.0 mg/ml of AC-11 (3 samples at each concentration; Anonymous, 2004a). After 72 hours, a 6.5%, 33.5%, 64.3%, and 86.3% decrease in mean human Jurkat leukemia cell counts (viable cells measured by coulter counter and compared to control; 0 mg/ml of AC-11) was observed in cultures exposed to 0.125, 0.25, 0.5, and 1.0 mg/ml of AC-11, respectively. Mean murine leukemia cell counts decreased 12.6%, 20.8%, and 90.5% in cultures exposed to 0.25, 0.5, and 1.0 mg/ml of AC-11, respectively. However, when murine leukemic cells (200,000 cells/0.2 ml) were transplanted intraperitoneally into athymic Swiss NCr-nu/nu mice that were subsequently treated with 0, 80, or 160 mg/kg of AC-11 for 7 days (7 animals/group), no improvement in survival time was observed in AC-11-treated animals (Anonymous, 2004a).

Human colon cancer cells (5,000,000 cells/0.2 ml) were transplanted subcutaneously into athymic Swiss NCr-nu/nu mice that were subsequently treated with 0 or 80 mg/kg of AC-11 for 30 days (8 animals/group; Anonymous, 2004a). Tumor growth (tumor volume, calculated by caliper measurements of tumor length and width; and tumor weight) was assessed twice a week. There were no substantial differences in AC-11-treated and untreated controls in tumor weight during and after AC-11 therapy.

Skin Whitening in Humans

AC-11 was applied topically, once daily for 7 – 10 days, to 10 female volunteers between 18 and 45 years of age with Fitzpatrick skin type I or II (Anonymous, 2004b). Excluded

from participation were women with acute or chronic diseases (including dermatological problems); those exhibiting sunburn, rashes, scratches, burn marks, excessive warts, nevi, moles, scars, active dermal lesions, etc. that might interfere with the evaluation of test results; those who were pregnant or lactating; and those being treated with systemic or local retinoids, antihistamines, and/or similar agents two weeks before and during the course of the study. The participants had three distinct areas on their backs, each approximately 4 cm^2 in size, reserved for the evaluation of skin whitening. Following exposure to a Xenon Arc Solar Stimulator (150 Watt) and baseline color measurements, one area was left untreated, one was treated with topical AC-11 (0.5%) and the third area was treated with kojic acid (2%). The skin whitening effect produced by AC-11, and calculated as the difference in the area under the curve of color measurements of the treated and untreated skin areas, was similar in AC-11-treated areas (Whitening Factor = 1.85) to that produced by kojic acid (Whitening Factor = 1.7).

AC-11 Safety

Animal Toxicology

Food consumption, weight gain and organ weight coefficients were assessed following multiple doses of AC-11 (Tables 9 and 10), as were acute signs of toxicity and histopathological changes. Lethality (LD_{50}) was measured following a single AC-11 dose.

Sheng et al, 2000a; Experiment H
Zero, 40, or 80 mg/kg/day doses of AC-11 were administered to 30 female Wistar-Furth rats (10 rats per dose; random allocation to treatment groups) by gavage for 8 weeks. No acute signs or symptoms of toxicity were observed. All groups of rats gained weight and no significant differences were observed in food consumption in AC-11-treated rats and controls.

Sheng et al, 2000a; Experiment I
Zero and 160 mg/kg/day doses of AC-11 were administered to 14-16 female Wistar-Furth rats (7-8 rats per dose) by gavage for 4 weeks. There were no significant differences in weight gain over the course of the study or in mean food consumption (g/day/rat) in AC-11-treated rats and controls.

Single doses of 0, 1, 2, 4, and 8 g/kg of AC-11 were administered by gavage to female Wistar-Furth rats, 5 rats at each dose (Sheng et al, 2000a). No deaths or signs of toxicity were observed in the animals for up to 2 weeks following the dose. It was thus concluded that the LD_{50} of AC-11 in rats is in excess of 8 g/kg.

Table 9. The effect of oral AC-11 on food consumption and weight in Wistar-Furth rats.

Experiment Study	n*	AC-11 dose (mg/kg/day)	Duration of Therapy	Comparison To	Outcome
H Sheng et al, 2000a	30	40 and 80	8 weeks	Untreated Controls	No significant difference in food consumption No significant difference in weight gain
I Sheng et al, 2000a	14 to 16	160	4 weeks	Untreated Controls	No significant difference in food consumption No significant difference in weight gain
K Sheng et al, 2000b	47	40 and 80†	15 days	Doxorubicin-treated Controls	No significant difference in weight

*The number of rats studied.
†AC-11 therapy was started 24 hours after the last of 3 intraperitoneal doses of doxorubicin.

Table 10. The effect of AC-11 on organ weight coefficients* in Wistar-Furth rats.

Experiment Study	n†	Dose (mg/kg/day)	Duration of Therapy	Outcome
J Sheng et al, 2000a	50	5 - 160	4-8 Weeks	No significant differences in liver, kidney, spleen, and heart weight coefficients compared with controls
K Sheng et al, 2000b	47	40 and 80‡	15 days	No significant differences in liver, kidney, and spleen weight coefficients compared with doxorubicin only controls Significantly increased heart weight coefficient compared with doxorubicin only controls

*Organ weight coefficients = organ weight/body weight x 100.
†The number of rats studied.
‡AC-11 therapy was started 24 hours after the last of 3 intraperitoneal doses of doxorubicin.

Sheng et al, 2000a; Experiment J

Animals exposed to various AC-11 doses (5 mg/kg to 160 mg/kg; n = 50) and durations of therapy (4 weeks or 8 weeks), and controls (n = 21) were sacrificed and organ weights obtained. Histopathologic examinations, conducted using a light microscope and a blinded examiner, were obtained on liver, kidney, spleen, and heart tissues. There were no significant differences in organ weight coefficients (organ weight/body weight x 100) in AC-11-treated groups and controls. Histopathologic examinations revealed no increased pathological changes such as necrosis, fibrosis, increased mitotic activity, and proliferation when AC-11 animals were compared to controls.

Sheng et al, 2000b; Experiment K

Daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered orally, after dissolution in sterile tap water, to 24 Wistar-Furth rats (12 at each dose; random allocation to treatment groups) 24 hours after the last of three 2 mg/kg intraperitoneal doses of doxorubicin (48 hours separating each doxorubicin dose). Two additional study groups were included in the experiment; in one group the animals received only doxorubicin (n = 12), while the other included untreated controls (n = 11). Body weight was significantly lower ($p < 0.05$) in doxorubicin-treated animals (in the group treated only with doxorubicin as well as in the 2 groups treated with AC-11) compared with untreated controls. However, no significant differences were noted in body weight in animals treated with doxorubicin only and doxorubicin plus AC-11 (at either 40 mg/kg or 80 mg/kg). The animals were sacrificed after 3 weeks and organ weights measured. No significant differences were noted in spleen, liver, or kidney organ weight coefficients (organ weight/body weight x 100) in doxorubicin only, doxorubicin plus 40 mg/kg of AC-11, and doxorubicin plus 80 mg/kg of AC-11. There was, however, a significant ($p < 0.05$) increase in the heart weight coefficient of animals that received doxorubicin plus 80 mg/kg of AC-11 (0.386% ± 0.034) when compared with doxorubicin only treated animals (0.369% ± 0.022). No significant differences were noted in heart weight coefficient in doxorubicin plus 80 mg/kg AC-11-treated animals when compared to untreated controls.

Female C57BL/6 mice (7 in each group) were fed AC-11 in drinking water at 0 and 4 mg/ml concentrations for 25 days (Åkesson et al, 2003b). No significant differences were

observed in total body weight or spleen weight of AC-11-treated or untreated controls.

Adverse Events in Humans

Five small studies have been conducted in which oral AC-11 was administered to humans. No adverse reactions were reported in 4 of the studies, summarized in Table 11, in which AC-11 was administered to an aggregate of 29 volunteers at doses of 350 mg to 700 mg per day for up to 8 weeks.

In a separate study which did not report adverse events, a summary of clinical data collected from physician interviews and self-reported case report forms indicated that after AC-11 therapy headache, diarrhea, and stress occurred more frequently than prior to therapy (Pero et al, 2005). Headache was reported in 3 and diarrhea in 5 of 14 volunteers (7 males and 7 females), between 52 and 64 years of age, before being treated for 4 weeks with 400 mg AC-11 per day in combination with 200 mg of nicotinamide, 20 mg of zinc, and 500 mg of KMA complex (composed of the following mushroom extracts: *Cordyceps sinensis*, *Grifola blazei*, *Grifola frondosa*, *Tramates versicolor*, and *Ganoderma lucidum*). After therapy, 5 volunteers reported headache and 6 reported diarrhea. The after therapy stress test level (method of assessment not reported) was greater after AC-11 than before (5.0 and 4.7, respectively; clinical significance of the numeric values was not reported). The increases noted in the incidence of headache and diarrhea, and the increased stress test level were not statistically significant.

Table 11. Adverse Events in Humans Treated With AC-11.

Reference	Population	n*	Daily Dose (mg)	Duration of Therapy	Adverse Events
Pero et al, 2002	volunteers between 35 and 55 years of age	5	350†	4 weeks	No side effects were observed or recorded that could be attributed to AC-11
Sheng et al, 2001	apparently healthy volunteers	8	250 or 350	8 weeks	There were no signs or symptoms of AC-11-induced adverse events
Lamm et al, 2001	male Caucasian volunteers between 40 and 60 years of age	11	700‡	60 days	No side effects were attributed to AC-11 supplementation
Sheng et al, 2000a	apparently healthy adult male volunteers between 32 and 58 years of age	4	350	6 weeks	No observed adverse reactions

* The number of AC-11-treated volunteers.
† A vitamin/mineral/antioxidant supplement was administered with AC-11.
‡ Pneumococcal vaccine was administered on day 30.

Clinical Chemistry and Hematology

Clinical Chemistry: Ten male Caucasian volunteers between 40 and 60 years of age, with no apparent acute diseases, received 700 mg of oral AC-11 (350 mg twice a day for 60 days (Lamm et al, 2001). Blood was obtained prior to AC-11 administration (Day 1) and on day 60 for assessment of various clinical chemistry parameters (Table 12). Mean values were not statistically different for most tests when before and after therapy comparisons were made. However, sodium decreased from 141 ± 0.6 to 139.7 ± 1.6 mmol/L ($p < 0.05$) and iron increased from 76.3 ± 24.3 to 105.4 ± 28.4 µg/dL ($p < 0.05$). Although normal ranges of clinical chemistry tests were not reported, it is likely that neither change resulted in an abnormal mean value (Wallach, 1978). The authors did not report if any of the individual chemistry values became abnormal after AC-11 therapy.

Twelve apparently healthy volunteers (6 males and 6 females between 28 and 54 years of age) were randomly assigned to receive either 0, 250, or 350 mg/day of AC-11 for 8 weeks (Sheng et al, 2001). Venous blood was collected prior to administration of AC-11 and after 8 weeks of therapy. In the case of volunteers who did not receive AC-11, blood was collected prior to when therapy would have begun and after 8 weeks. Blood samples were analyzed for total protein, albumin, glucose protein, iron, sodium, potassium, calcium, magnesium, triglycerides, and cholesterol (Table 13). The mean post-AC-11 therapy sodium concentration was significantly ($p < 0.05$) decreased (before therapy: 141.3 ± 1.0 mmol/L; after therapy: 140.3 ± 0.8 mmol/L) in volunteers who received 350 mg/day. As in the study of Lamm et al (2001) normal ranges of clinical chemistry tests were not reported; however, it is unlikely that the observed statistically significant change resulted in an abnormal mean value (Wallach, 1978). The authors did not report if any of the individual chemistry values became abnormal after AC-11 therapy.

Table 12. Clinical Chemistry Values Obtained Before and After 60 Days of AC-11 Therapy in 10 Volunteers With No Apparent Acute Diseases (Lamm et al, 2001).

Clinical Chemistry Test	Significant Change*
Sodium	Yes*
Potassium	No
Calcium	No
Iron	Yes†
Chloride	No
Blood Urea Nitrogen	No
Creatine‡	No
Blood Urea Nitrogen/Creatine‡ Ratio	No
Uric Acid	No
Glucose	No
Total Protein	No
Albumin	No
Globumin [sic]	No
Albumin/Globulin Ratio	No
Cholesterol	No
HDL Cholesterol	No
Cholesterol/HDL Cholesterol Ratio	No
LDL Cholesterol	No
Triglycerides	No
Total Bilirubin	No
Direct Bilirubin	No
Alkaline Phosphatase	No
γ-Glutamyl Transpeptidase	No
Aspartate Aminotransferase	No
Alanine Aminotransferase	No
Lactic Dehydrogenase	No

* Significant ($p < 0.05$) difference in mean sodium concentration before AC-11 therapy (141.0 ± 0.6 mmol/L) and after AC-11 therapy (139.7 ± 1.6 mmol/L).
† Significant ($p < 0.05$) difference in mean iron concentration before AC-11 therapy (76.3 ± 24.3 µg/dL) and after AC-11 therapy (105.4 ± 28.4 µg/dL).
‡ It is likely that the authors actually determined Creatinine concentrations and Blood Urea Nitrogen/Creatinine ratios.

Table 13. Clinical Chemistry Values Obtained Before and After 8 Weeks of AC-11 Therapy in Apparently Healthy Volunteers Who Received 250 mg/day (n = 4) or 350 mg/day (n = 4), And in Apparently Healthy Volunteers Who Did Not Receive AC-11 Therapy (n = 4; Sheng et al, 2001).

Clinical Chemistry Test	Significant Change
Total Protein	No
Albumin	No
Glucose Protein	No
Iron	No
Sodium	Yes*
Potassium	No
Calcium	No
Magnesium	No
Triglycerides	No
Cholesterol	No

* Significant ($p < 0.05$) difference in mean sodium concentration before AC-11 therapy (141.3 ± 1.0 mmol/L) and after 350 mg/day of AC-11 therapy (140.3 ± 0.9 mmol/L).

Hematology: A total of 12 male Caucasian volunteers between 40 and 60 years of age, with no apparent acute diseases, received 700 mg of oral AC-11 (350 mg twice a day) for 60 days (Lamm et al, 2001). On day 30 the volunteers were given 0.5 ml pneumococcal vaccine, intramuscularly. Blood samples were obtained on day 0 (before AC-11 administration) and on day 60, and sent to a commercial laboratory for analysis of various hematologic parameters (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, mean platelet volume, total platelet count, total and percent polymorphonuclear neutrophils, total and percent lymphocytes, total and percent monocytes, total and percent eosinophils, total and percent basophils, total and percent CD3 lymphocytes, total and percent T-helper lymphocytes, total and percent T-suppressor lymphocytes, T-helper/T-suppressor ratio; Table 14). Only the change in polymorphonuclear neutrophils (before = 4084 ± 1121 mm^3;

Table 14. Hematology Values Obtained Before and After 60 Days of AC-11 Therapy in 12 Volunteers With No Apparent Acute Diseases (Lamm et al, 2001).

Hematology Test	Significant Change
Total White Blood Cells	No
Total Red Blood Cells	No
Hemoglobin	No
Hematocrit	No
Mean Corpuscular Volume	No
Mean Corpuscular Hemoglobin	No
Mean Corpuscular Hemoglobin Concentration	No
Red Blood Cell Distribution Width	No
Mean Platelet Volume	No
Total Platelet Count	No
Total Polymorphonuclear Neutrophils	Yes*
Percent Polymorphonuclear Neutrophils	No
Total and Percent Lymphocytes	No
Total and Percent Monocytes	No
Total and Percent Eosinophils	No
Total and Percent Basophils	No
Total and Percent CD3 Lymphocytes	No
Total and Percent T-Helper Lymphocytes	No
Total and Percent T-Suppressor Lymphocytes	No
T-Helper/T-Suppressor Ratio	No

* Significant ($p < 0.05$) difference in mean polymorphonuclear neutrophil count before AC-11 therapy (4084 ± 1121 mm^3) and after AC-11 therapy (3243 ± 836 mm^3).

after = 3243 ± 836 mm^3) achieved statistical significance ($p < 0.05$).

Twelve apparently healthy volunteers (6 males and 6 females between 28 and 54 years of age) were randomly assigned to receive either 0, 250, or 350 mg/day of AC-11 for 8 weeks (Sheng et al, 2001). Venous blood was collected prior to administration of AC-11 and after 8 weeks of therapy. In the case of volunteers who did not receive AC-11, blood was collected prior to when therapy would have begun and after 8 weeks. Blood samples were analyzed within one hour of collection by an automated hematology analyzer for total

red and white blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, total and percent lymphocytes, total and percent monocytes, total and percent neutrophils, and lymphocyte ratio (not defined; Table 15). Before therapy and after therapy mean corpuscular hemoglobin concentrations were significantly ($p < 0.05$) different among groups (0, 250, and 350 mg/day). It cannot be stated with any certainty whether the differences could be clinically significant as the values reported for all groups before and after therapy were not physiologic. Volunteers who received 350 mg/day had a significantly ($p < 0.05$) decreased mean platelet count (before therapy: 223 ± 50 $10^3/mm^3$; after therapy 201 ± 53 $10^3/mm^3$). Normal ranges of hematology tests were not reported; however, it is unlikely that the observed statistically significant change resulted in an abnormal mean value (Wallach, 1978). The authors did not report if any of the individual hematology values became abnormal after AC-11 therapy.

Table 15. Hematology Values Obtained Before and After 8 Weeks of 0 (n = 4), 250 (n = 4) or 350 mg/day (n = 4) of AC-11 in Apparently Healthy Volunteers (Sheng et al, 2001).

Hematology Test	Significant Change
Total White Blood Cells	No
Total Red Blood Cells	No
Hemoglobin	No
Hematocrit	No
Mean Corpuscular Volume	No
Mean Corpuscular Hemoglobin	No
Mean Corpuscular Hemoglobin Concentration	Yes[1,2]
Red Blood Cell Distribution Width	No
Total Platelet Count	Yes[3]
Total and Percent Lymphocytes	No
Total and Percent Monocytes[4]	No
Total and Percent Neutrophils[5]	No
Lymphocyte Ratio[6]	No

[1] Significant ($p < 0.05$) difference in mean corpuscular hemoglobin concentration among groups before AC-11 therapy (0 mg/day = 347 ± 3%; 250 mg/day = 358 ± 4%; 350 mg/day = 345 ± 10%). The reported values are obviously erroneous. They are probably ten times the actual values (normal mean corpuscular hemoglobin concentration in adult males and females = 34 ± 2% [Wallach, 1978]).

[2] Significant ($p < 0.05$) difference in mean corpuscular hemoglobin concentration among groups after AC-11 therapy (0 mg/day = 342 ± 2%; 250 mg/day = 355 ± 5%; 350 mg/day = 344 ± 9%). The reported values are obviously erroneous. They are probably ten times the actual values (normal mean corpuscular hemoglobin concentration in adult males and females = 34 ± 2% [Wallach, 1978]).

[3] Significant ($p < 0.05$) difference in mean platelet count before (223 ± 50 $10^3/mm^3$) and after (201 ± 53 $10^3/mm^3$) 350 mg/day of AC-11.

[4] Assumed to be monocytes; however, the authors did not define the acronym used (MXD) in the tabulation of their data. The text of the report indicated that there were no significant differences in numbers/percentages of monocytes.

[5] Assumed to be neutrophils; however, the authors did not define the acronym used (NEU) in the tabulation of their data.

[6] Not defined by authors.

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