THE ROLE OF ANTIOXIDANTS IN DERMATOLOGY

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Abstract :

Oxidative stress is exerted on the skin by endogenous and exogenous pathways. Solar energy is transferred to the skin by visible light and ultraviolet light, and represents the most challenging exogenous oxidative stress. The antioxidant defense mechanisms of the skin are beyond the required standard compared to other organs as liver, muscles and kidneys. Oxidative stress possibly shares in the aetiopathogenesis of some skin disorders as ageing, progeria, photoageing, photocarcinogenesis and few inflammatory disorders. Therapeutic intervention by antioxidants was done both experimentally and clinically using many products given by oral route or applied directly to the skin. Assessment of the oxidative states is helpful in diagnosis and therapy. Therapeutic trials of antioxidants in neutrophilic and eosinophilic dermatoses may be the target of future research.

THE OXIDATIVE STRESS

Over 90% of the universe is composed of hydrogen, 7% of helium and the remaining is composed of the other elements. Oxygen is the third more abundant in the universe and the most abundant element in the earth crust (53%). Because hydrogen is active and the most abundant element, it reacted with oxygen to form water, with carbon to form methane and with nitrogen to form ammonia. Molecular oxygen was first found in the earth's atmosphere two billion years ago after its dissociation from water by algae via photosynthesis. The inspired oxygen is used for extracting energy from ingested organic materials during coupled phosphorylation, 99% of the of that oxygen end in water or carbon dioxide, however, the remaining one percent is chemically transformed into reactive species which might serve a minor physiological function and a major pathological mediator[1].

There are four known reactive oxygen species (ROS): the singlet oxygen, the superoxide, the hydrogen peroxide and the hydroxyl radical. Singlet oxygen is formed when one electron in the outermost orbit in the oxygen atom moves into a higher energy level and changes its spin, this makes the less reactive molecular oxygen more reactive (fig 1). Singlet oxygen has two choices either to transfer such energy to a nearby organic substance or to continue creating more aggressive oxygen species. Singlet oxygen is continuously formed in the skin as a result of exposure to ultraviolet light, and consequently it is considered the major source of oxidative stress in the skin[2,3]. Superoxide radical is formed when one electron is added to the oxygen atom, if another electron is added two oxygen atoms and two hydrogen atoms will form hydrogen peroxide, which has a relatively long life span (up to 10 seconds), that gives it a good opportunity to react with the surrounding structures. Finally if one more electron is added hydroxyl radical, the most powerful oxidant known to science is formed[4].

Reactive oxygen species in the presence of transitional metals are quite harmful, intermediate reactive

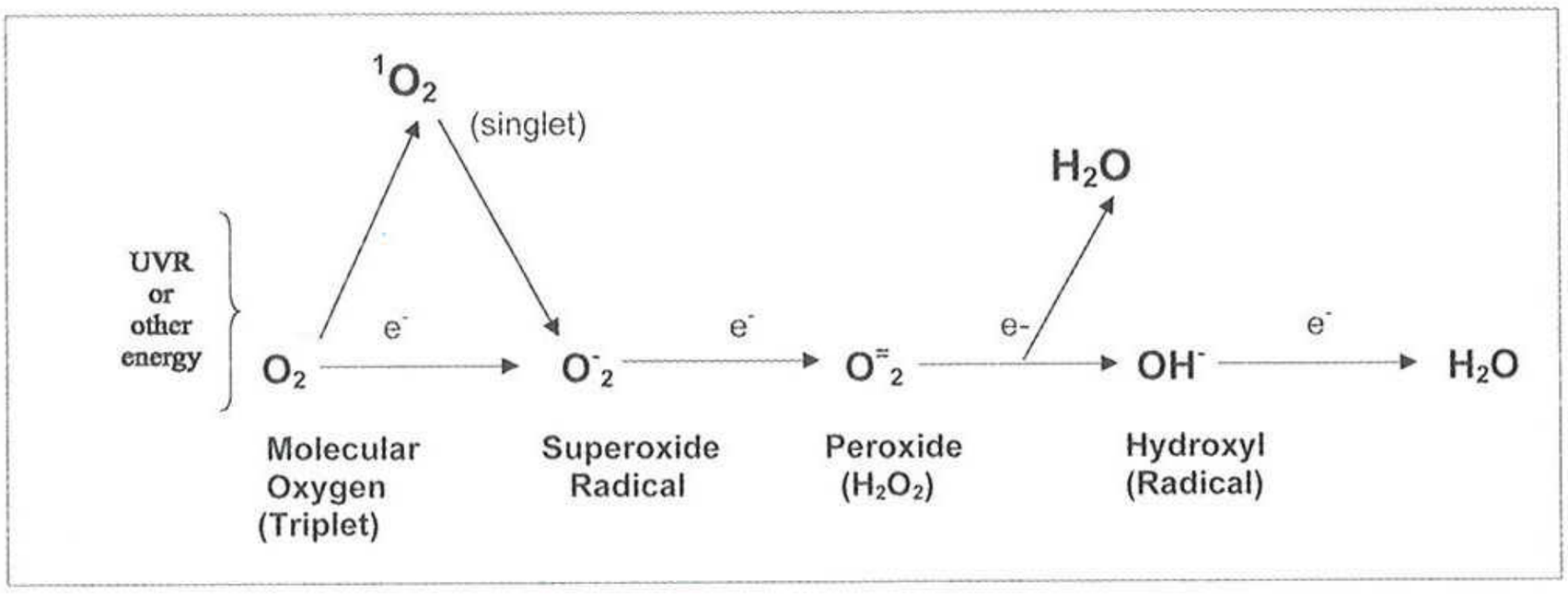

FIG 1 SHOWING THE OXYGEN ATOM AND FORMATION OF REACTIVE OXYGEN SPECIES AND OXYGEN MOLECULE

species (ROI) are highly active and responsible for most of the drawbacks of oxidative stress. Reactive nitrogen species (RNS) are recently recognized, they share some of the pathways that occur during oxidative stress. Nitric oxide is a free radical synthesised by nitric oxide synthetases (NOSs) in the endothelium and neurones. Two other reactive nitrogen species are described the peroxynitrite and dinitrotrioxide , the first is more important in biochemical pathways. The production of reactive nitrogen species may be helpful to the body, however, this is dependent on the base line oxidative state. Nitric oxide may even act as antioxidant and nullifies the effects of reactive oxygen intermediates formed by transitional metals as iron and copper[5].

AT THE MOLECULAR LEVEL

Under ordinary physiological conditions lipid peroxidation is going on to release fatty acid essential for multiple pathways and cell signalling. Lipid peroxidation involves first the lipids with high double bond concentration which is more vulnerable to oxidative process. The first step in lipid peroxidation is hydrogen subtraction that converts the lipid into a conjugated diene, a free radical which if not quenched by an antioxidant the reaction will continue unchecked. If conjugated with an oxygen molecule a very active peroxylipid radical is formed which is now ready to attack another lipid molecule and run a chain of the viscous cycle of lipid peroxidation. Being the most important component of the cell membrane, lipid peroxidation affects both the integrity and permeability of the cell membranes[6,7] (fig 2)

The ability of the body to repair oxidised proteins is limited to the sulphur containing proteins. Instead, the damaged proteins are liable to degeneration of amino acids constituents by the action of various endogenous proteases including: cathepsin c, calpain, trypsin and the 20s proteosome whose activity is under the effect of diverse regulatory factors[8].

Oxidation of proteins is intensified in the presence of transitional metals as iron and copper that form alkylperoxides which are capable of reacting with any of the known organic materials. Peroxynitrite can modify tyrosine, tryptophan, cysteine and methionine residues of proteins. Protein oxidation affects structural and functional proteins, the oxidation of structural protein renders them more friable, fragile and lose their configuration. Collagen composes 90% of the dermal protein, free radicals play an important role in collagen synthesis with vitamin C as a catalyst. Except for ascorbic acid antioxidants have an antifibrotic activity. Functional proteins show more serious reactions on oxidation as they act as enzymes and cell signalling factors which may explain the mass failure of body systems in severe oxidative attacks[8,9] (fig 3)

Cabohydrate residues as glycoproteins and glycolipids constitute a considerable quantity of the dermal ground substance, while in the epidermis it is a major desmsomal contributor and cell membrane. It plays an important role in cell differentiation , interaction and signalling.(fig 4) These molecules are subject of oxidative attacks which alter their function[7]

Nucleic acids are subjected to more than 10,000 oxidative hits per cell per day!!. The remarkable damage repair in the body systems is highly competent in most instances. If these protective mechanisms are disabled DNA damage occurs in the form of strand breaks, cross linking and base modification.

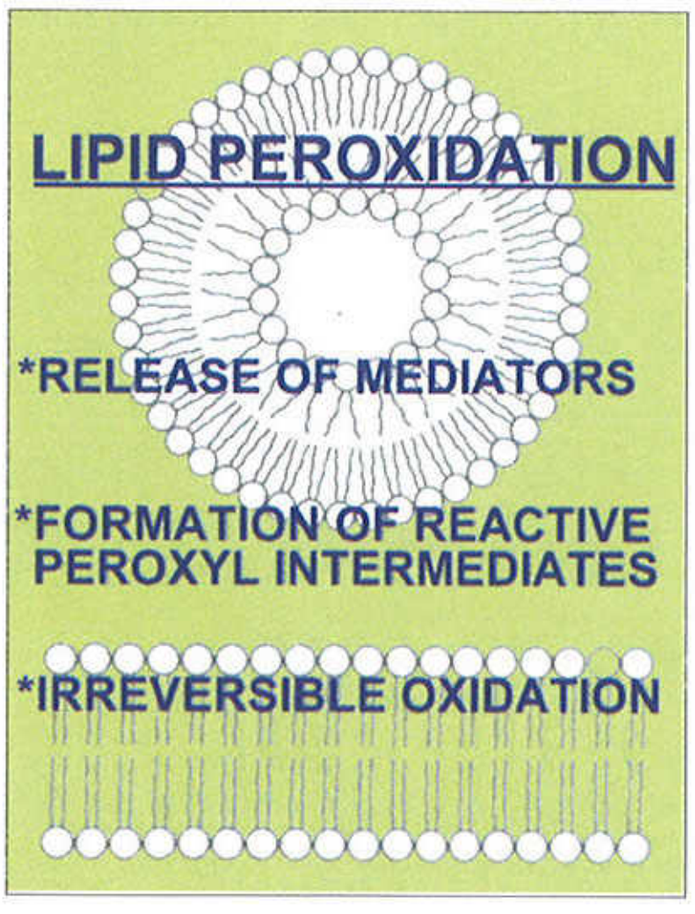

FIG 2 LIPID PEROXIDATION

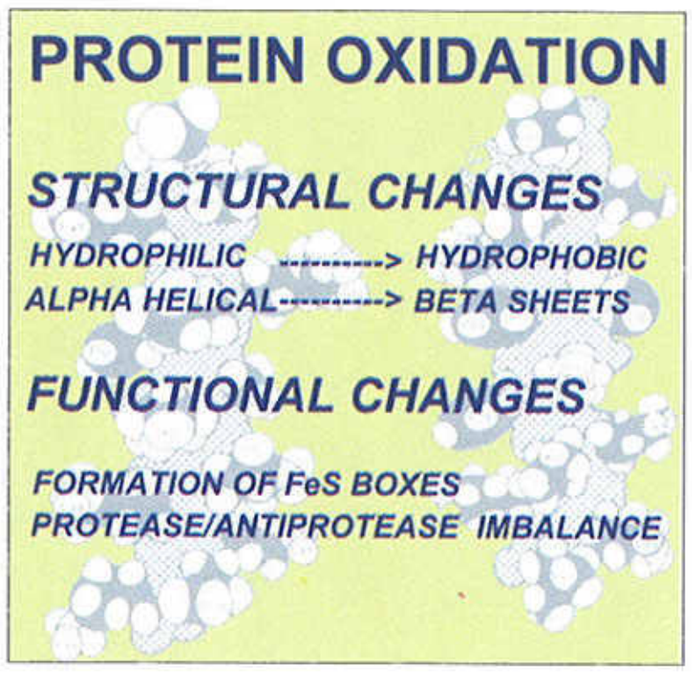

FIG 3 PROTEIN OXIDATION

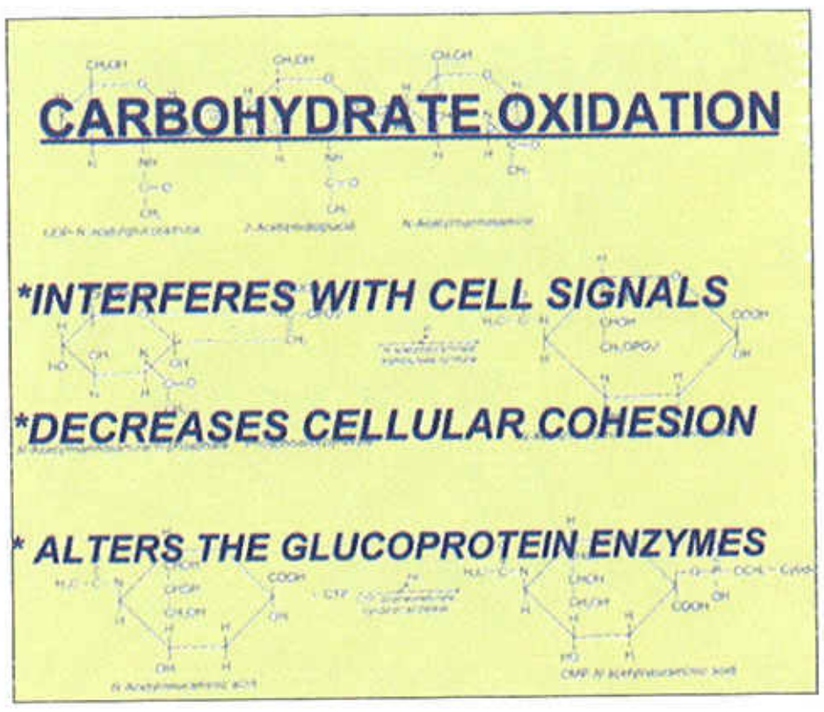

FIG 4 CARBOHYDRATES OXIDATION

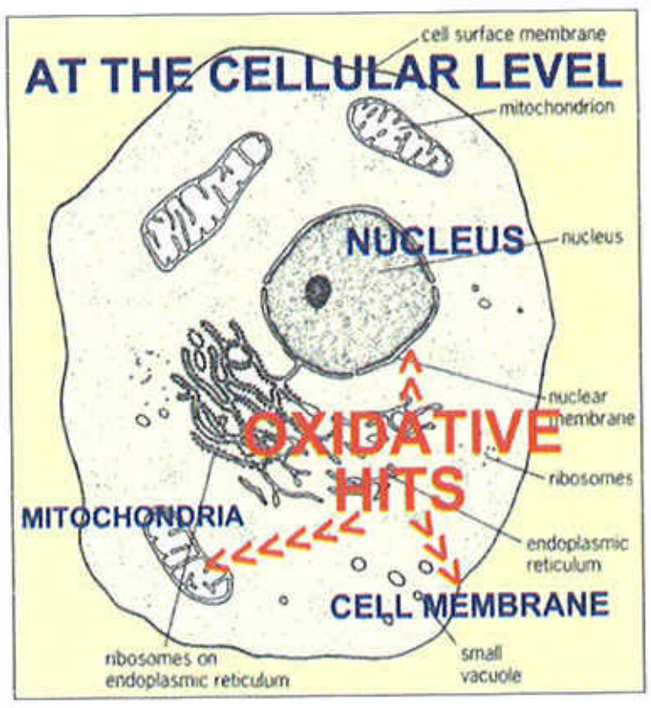

FIG 6 OXIDATIVE TARGETS IN THE CELLS

Added to the physiological stress oxidative of the body, the oxidative stress caused by ultraviolet rays on the exposed parts of the epidermis, UVB has more energy than UVA and its destructive effect on DNA is more pronounced[9,10]. (fig 5)

AT THE CELLULAR LEVEL:

Mitochondria is the source of cellular energy and eventually the most susceptible to oxidative hits, superoxide and hydrogen peroxides are formed mainly in the mitochondria. Hydrogen peroxide has the Iongest life span among the other radicals, which may reach up to 10 seconds, this period is very long on the molecular scale and gives the chance to hydrogen peroxide to cross membranes and react with most of the cellular components. On the other hand the hydroxyl radical though owns the highest affinity and reactivity among oxygen species, it has the shortest life span, so its effects are almost localized

FIG 5 DNA OXIDATION

[1,11] (fig 6)

Singlet oxygen is continuously formed as a result of skin irradiation in most of the oxygen containing compounds all over the cell, it has a longer life span than superoxide, and hence has the chance to cross a considerable distance on the molecular scale, and has the chance to attack membrane lipids and nuclear contents including nucleic acids. Superoxide is produced in the mitochondria, its detrimental effect is limited to that location ,however, it can propagate by generation of other oxidative species [12]

At the cellular level the antioxidant defences are present in the cell membrane (tocopherols), mitochondria (superoxide dismutase & perioxidases), cytoplasm (catalases) and in the nucleus (thioredoxin). While in the extracellular space most of the plasma antioxidants are present including ascorbic acid.[2]

AT THE TISSUE LEVEL

The epidermis is 90% cellular, while the dermis is only 20% cellular, it is evident that the epidermis for this reason is subjected to more endogenous oxidative stress; the lower part of stratum malpighii is more active metabolically and contains higher number of mitochondria than the upper part. Added the fact that billions of photons are traversing the epidermis daily from outside light and UVR. If also taken into consideration that the epithelial tissues though acquiring a moderate capacity to regenerate, they possess the modest antioxidant mechanisms compared to vital organs as liver and kidneys and the concentration of hydrophilic natural antioxidants (uric acid, ascorbate and glutathione) show a gradual decrease from the basal layer to the stratum corneum [13,14] Therefore, we can conclude that the epidermis is one of the most susceptible tissues to oxidative stress in the whole body.(fig 7)

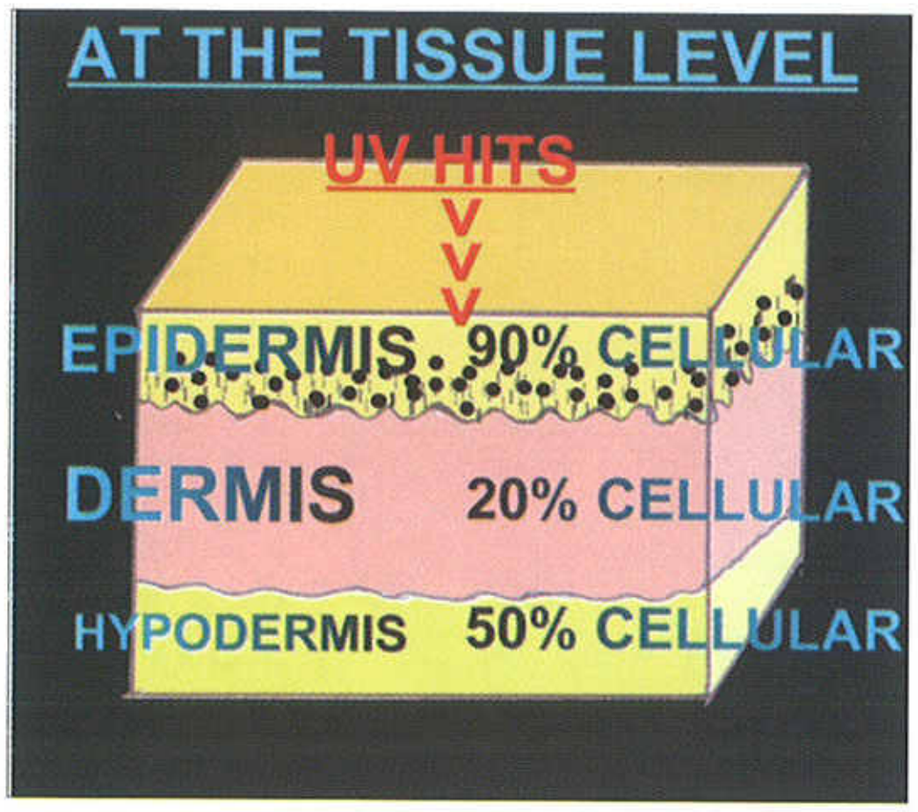

FIG 7 OXIDATION AT THE TISSUE LEVEL

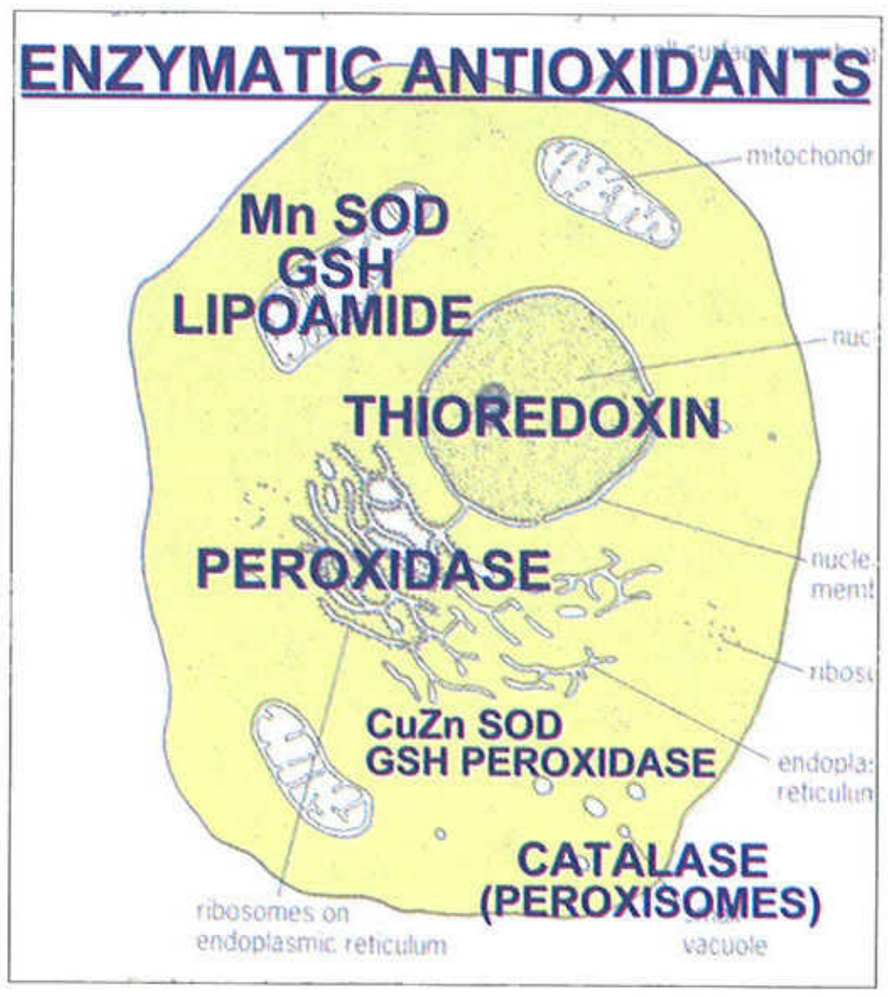

FIG 8 DIFFERENT ANTIOXIDANT ENZYMES

THE SKIN's ANTIOXIDANT SYSTEMS

How the skin protects itself from the damaging effects of the oxidative species?. A full understanding of these protective mechanisms requires more research on this issue because most of the work in literature was done on animals particularly the hairless mice. A key concept in appreciating the effects of oxidative radicals is that any injury capable of producing an inflammatory response will create oxidative damage. Any skin disorder, regardless of the aetiology, that is characterised by inflammation will have a component of oxidative damage and therefore theoretically will benefit from antioxidant therapy. The skin protective mechanisms against oxidative stress are generally classified as enzymatic and nonenzymatic systems.

THE ENZYMATIC ANTIOXIDANT SYSTEM

The enzymatic antioxidant systems identified in the skin are: superoxide dismutase, catalase, peroxidases, glutathione system, thioredoxin system, and lipoamide system.(fig 8)

Superoxide dismutase (SOD) is a metal-containing enzyme that has at least three known forms . In human the copper-zinc SOD is present in the cytoplasm, and the manganese SOD is found in the mitochondrial membrane, while the iron containing SOD is not found in human. Estimates of epidermal level of SOD vary from 5-10 times lower than that of the liver, kidneys and muscles, while the fatty tissue is 80 times lower. Its main action is to convert hydrogen peroxide to water and molecular oxygen[15]. Catalase is one of the most studied enzymes in all times, and is one of the oldest known enzymes. It has a heme portion in its molecule. Its activity is highest in the subcutis, in the cell it is not found in the mitochondria being only found in the perioxisomes in the cytoplasm. Catalase converts hydrogen peroxide into water and molecular oxygen, though it has a low affinity for the substrate the reaction capacity is high[16].

Peroxidases are enzymes that decompose various peroxides (not only hydrogen peroxide). In the skin it is found in the non-keratinized cells. Peroxidase activity is also detected in fibroblasts, mast cells and macrophages Glutafhione system is receiving a growing attention in the scientific community because it is considered the major antioxidant in the human body. It is found in both the epidermis and dermis. It is the first line of defence against oxidative attacks, it acts before catalase but it may be overwhelmed at times requiring catalase to provide assistance. Though it is highly useful it has a limited ability to interrupt membrane lipid peroxides. It is present in the cytoplasm and in the matrix of mitochondria. The glutathione system is composed of three enzymes: glutathione peroxidase, glutathione reductase and the glutathione transferase;

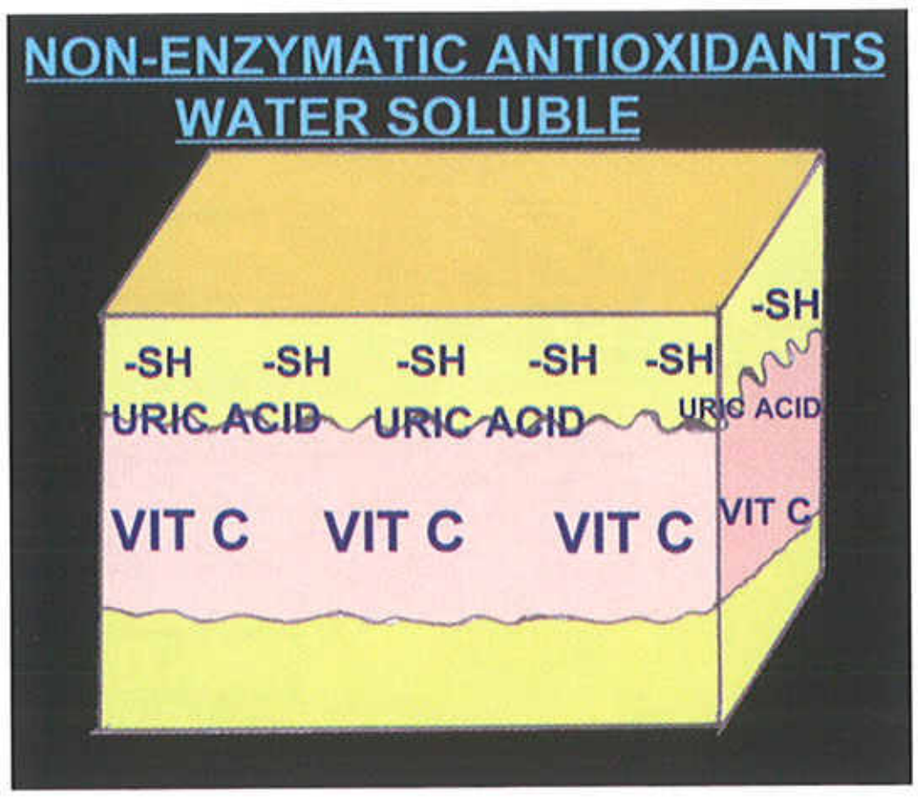

FIG 9 THE NONENZUMATIC FAT-SOLUBLE ANTIOXIDANTS

and three amino acids (glutathione): glycine, cysteine and glutamic acid, in conjunction with selenium and NADPH. Glutathione is the key compound in the system which can act as an antioxidant apart from the system by nonenzymatic reactions[17]

Thioredoxin reductase system acts like glutathione and contains also an SH- group, it is composed of thioredoxin, thioredoxin reductase and NADPH. It is present in high concentration in the epithelial tissues. Langerhans' cells and melanocytes contain both thioredoxin and thioredoxin reductase, while keratinocytes contain thioredoxin only. The system is present in both the cytosol and membrane bound as well as the mitochondria and nucleus. It shares in the transformation of ribonucleosides into deoxyribonucleosides and protects the cell from hydrogen peroxide attacks[18].The lipoamide system is a constituent of the mitochondrial dehydrogenase complex. Lipoamide is a form of lipoic acid which is attached to a lysine side chain of the enzyme dihydrolypoyl transacetylase, lipoamide dehdrogenase and NADH. The lipoate can scavenge singlet oxygen and reduce consumed (oxidised) thioredoxin[19].

THE NONENZYMATIC ANTIOXIDANT SYSTEM

The water soluble group consists of three types of compounds: the thiol containing compounds, ascorbic acid and urates. The most important to the skin is the thiol containing compounds. The hydroxyl radical is inactivated by thiol residues especially the glutathione. Skin contains relatively low amounts of ascorbic acid, about 41 microgram per gram dry weight and present mainly in the dermal extracellular tissue. Urates and uric acid are powerful radical scavengers especially the peroxyl and alkoxyl radicals. Allantoin is generated when free radicals attack uric acid, so the amount of allantoin in the body is a good indicator of free radical activity. The human skin contains relatively small amounts of uric acid about one third the plasma level[20] [Fig.9].

The fat soluble nonenzymatic antioxidants are vitamins A&E and ubiquinone (Co Q). Vitamin A and its precursor carotenoids have been used in medicine for a long time. Beta carotene is a powerful free radical scavenger, it is more effective than vitamin E. Though its plasma

concentration is 10-100 folds lower than vitamin E it is almost equal in their antioxidant activity. The skin concentration of both vitamin A and beta carotene ranges from 1.3-2.2 uglmg protein in the epidermis, while in the dermis the concentration of vitamin A is higher than beta carotenes (393 ug/mg Vs 0.775 ug/mg protein). There are more than 20 membrane receptors for vitamin A and its metabolites which reflects the importance of this vitamin to body systems that is far beyond the antioxidant activity[21].

Tocopherols are studied as early as 1934, it was chemically identified four years later and then widely used in industry since then as a major antioxidant. It was only re-evaluated in medical use after the discovery of its benefit to retrolental fibroplasia of prematures. Vitamin E is a mixture of eight tocopherols, all of them are biologically active, but alpha tocopherol is the most important. It is an integral part of biological membrane and considered the most important antioxidant for fat and fat-containing structures. In cell membrane the ratio of vitamin E to polyunsaturated phospholipids is 1: 1000 so its concentration would not provide an ultimate protection. The skin also contains low concentration of vitamin E ranging from 189-675 ng/mg protein[22].

Ubiquinone and ubipuinol or coenzyme Q (CoQ), the first is a ketone or the oxidized form, while the second is an alcohol or the reduced form. Both are extremely important electron acceptors that transfer electrons from flavins to cytochromes in the mitochondria. Ubiquinol protects the mitochondria against oxidative hits[23]. [Fig.10].

DERMATOSES POSSIBLY INDUCED BY OXIDATIVE PROCESSES:

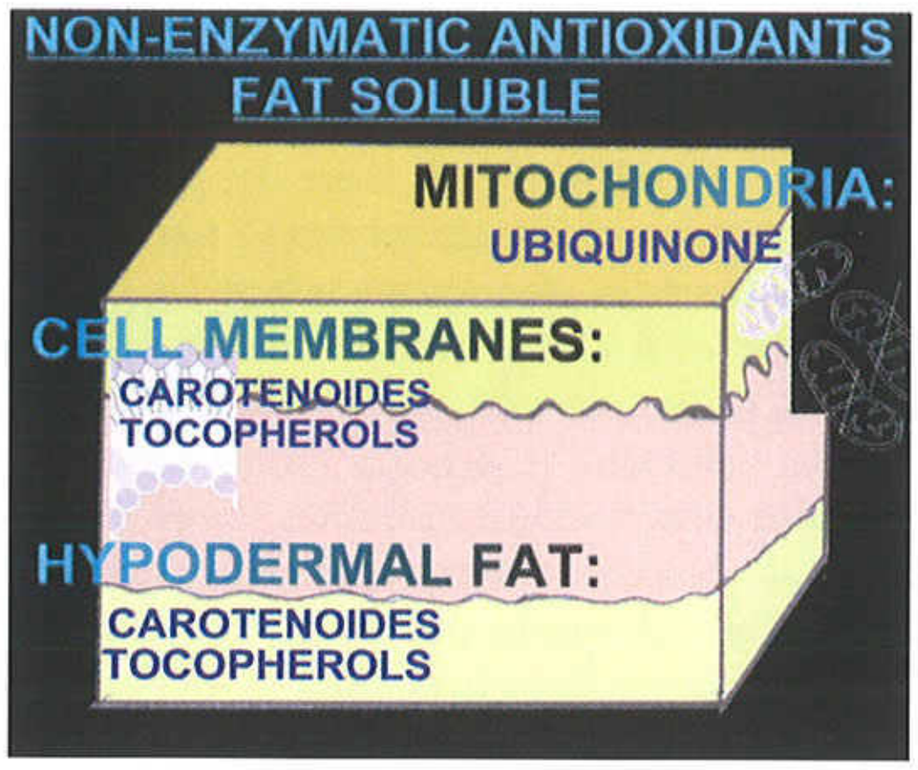

FIG 10 THE NONENZYMATIC WATER-SOLUBLE ANTIOXIDANTS

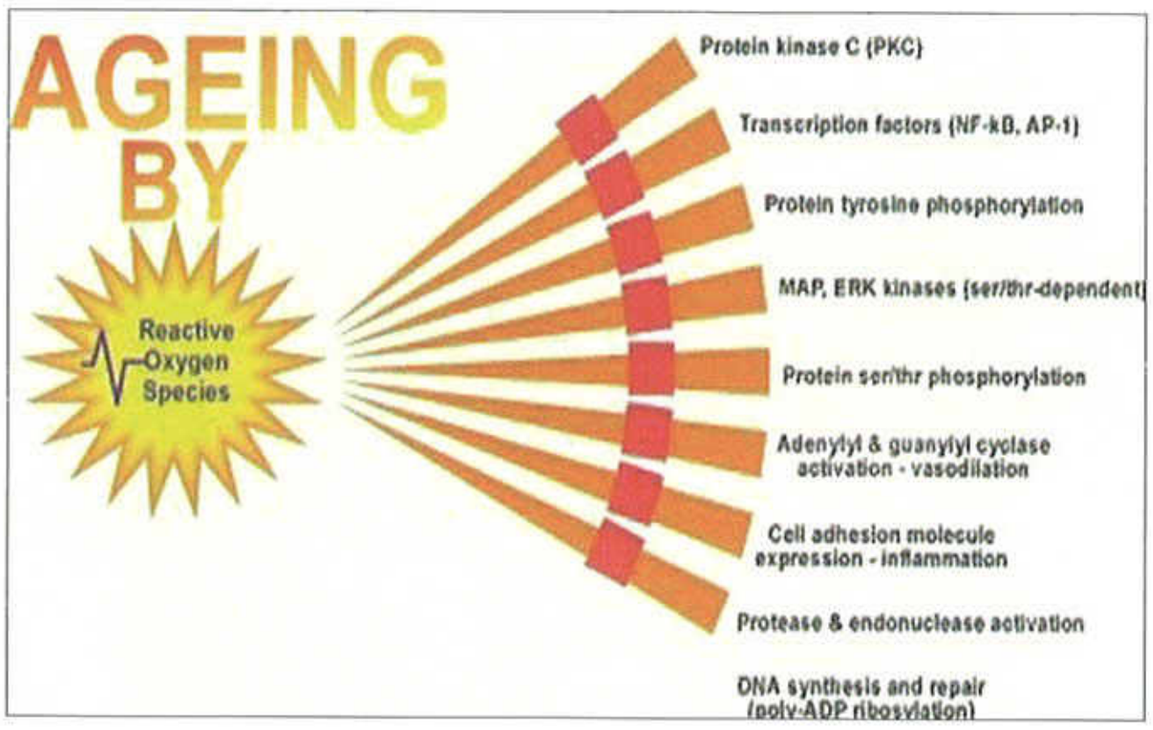

FIG 11 SHOWING THE DIFFERENT MECHANISMS CONTRIBUTED TO AGEING PROCESS

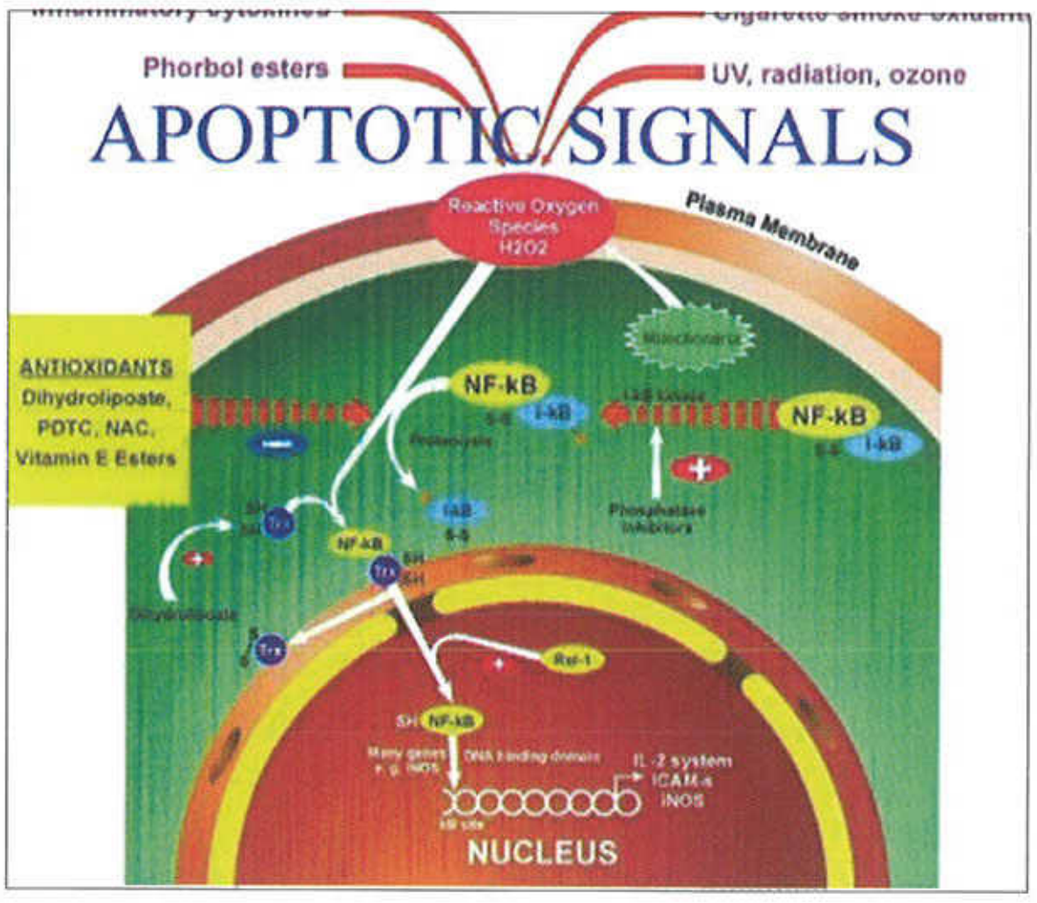

FIG 12 THE PATHWAY OF APOPTOTIC SIGNALS

AGEING AND PROGERIA

For decades , it was the man's dream to survive longer; recently, scientists focused on the pathogenesis of ageing process, how and why it happens?! Many researches where conducted , the unsolved issue was whether this ageing process is genetically coded or environmentally induced. In fact both views has its background but neither can supervene exclusively.

Numerous theories address specific ageing processes ranging from the molecular to the organ system level of function. (fig 11) The somatic mutation theory states that cells accumulate oncogenic mutations on a sporadic and scattered basis during the individual life span and that these mutations are of great importance to the age related impairment in the regenerative capabilities of cells. Programmed cell death (apoptosis) , immune dysregulation and hormones as melatonin and dehydroepiandrosterone were involved in ageing with more or less baseline evidence of their contribution[24]. (fig 12)

Free radical theory postulates that endogenously generated and highly reactive free radicals can induce somatic mutations, programmed cell death and play a role in age related immune dysregulation. Free radicals are supposed to play a sigirificant role in ageing process, accelerated skin ageing has been observed in some diseases characterised by an increased cellular free radical status as Werner syndrome and Down's syndrome[25]. The mutation of the mitochondrial genes is closely responsible for ageing processes. Mitochondria is the major source of perverted reactive oxygen species production. In one study on genetically modified Drosophilia the life span clearly increased when superoxide dismutase production is higher compared to non-modified strains[26]. In one in-vitro study the hairless mice epidermal cells showed decreased activity of glutathione perioxidase activity in ageing epidermal cells. Oxidative stress activates also the MitogenActivated Protein Kinase (MAPK) and Extra cellular signal-

Regulated Kinase (ERK) which plays a role in ageing and carcinogenesis[27]

Skin ageing is a complex biologic process affecting various layers, dermal changes are more conspicuous because it affects the extracellular matrix

and fibres which constitute about 80% of the dry weight of the skin.

The intrinsic ageing mechanisms are effective in every organ and occurs in both sun-exposed as well as sun-protected skin [28].

Syndromes of progeria are multiple, not essentially associated with photosensitivity or major metabolic disorders as diabetes. Protein oxidation is a common feature of these syndromes. Several mechanisms are involved in the protein oxidation: increased intracellular level of oxidised proteins, increased carbonyl residues in protein as in the lens and neurones; and the last is the altered enzymatic functions [29].

PHOTOAGEING AND PHOTOCARCINOGENESIS

The ageing changes caused by endogenous oxidative stress may occur in any organ, however, because skin is exposed to external environment, we cannot evaluate ageing changes without taking into consideration the effect of outside environment. The effect of ultraviolet rays on skin are studied extensively in the last decade, especially the photoageing effect. If we assume that some evidence may prove that oxidative stress plays a role in ageing, is this effect in simple linear cumulative or there may be specific pathway triggering?. Apoptois is induced by UVA, in addition to the documented damage of UVB on the nuclear DNA [30,31].(fig 13)

Photodamaged skin is characterised by wrinkles, laxity , uneven pigmentation and increased fragility. The photoaging effect of ultraviolet rays is doubtless, however ' , the mechanism by which it works is not yet clear, how the photons work on the molecular level ? A growing body of evidence that reactive oxygen species are generated by ultraviolet rays leading to enhanced oxidative damage in chronically sun-exposed skin. Increased oxidative stress may lead also to increased mitochondrial and nuclear DNA damage acclerating cellular ageing on one hand and contributing to the development of skin cancer on the other hand[32].

A principal difference between intrinsically aged skin and photoaged skin is the increased incidence of developing malignancies in the latter. This is probably due to excessive mutations in either oncogenes or tumour suppressor genes which are more prevalent in photodamaged skin. Mutations of p53 were also

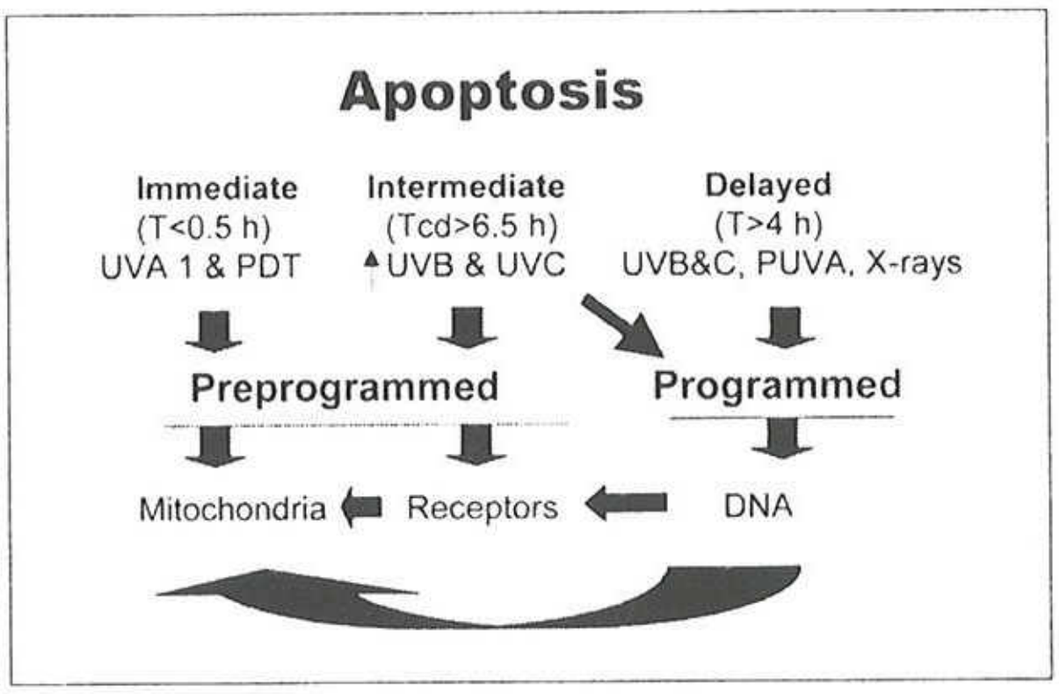

FIG 13 TYPES OF UV INDUCED APOPTOSIS

found excessively at the dermoepidermal junction located with frank accentuation at the UVR "hotspots" or the dipyrimidine sites. Another difference between the intrinsically aged skin and photoaged skin is the higher configurational changes of protein structure in the photoaged skin due to excessive protein oxidative attacks by UVR [33].

The skin cancer is the most prevalent form of cancer in humans and typically occurs in the sundamaged skin. The incidence of non-melanoma skin cancers is substantially increasing over the last few decades, in the US alone new cases are between 600 000 and 1 200 000 annually i.e. one third of the newly diagnosed cancers in US. This is attributed to UV-generated ROS as a drawback of ozone layer depletion. Oxidative stress by UVR induces a rapid and transient expression of growth response -1 gene which in turn stimulates a series of growth factor receptors. The reaction is mediated via reactive oxygen intermediates (ROI), and blocked by the presence of antioxidants. Stimulation of cellular growth in addition to the distortion of the tumour-checking genes as p53 will eventually run the cascade of neoplastic formation [34,35].

Xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy are characterised by increased sensitivity to UV-induced DNA damage and defective repair mechanisms. Only Xeroderma pigmentosum showed increase incidence of photocarcinogenesis. Oxidative species-induced photocarcinogenesis has multiple causes, that can be concised in two points, the development of abnormal genes which promote aberrant cell growth and the loss of checking anti-tumour genes as p53 mutations. Pyrimidine dimers which results from UV-induced oxidative stress may hinder

the genes encoding the excision-repair system. Cysteine residues attacked by oxy-radicals prevent the full transcriptional activity of strategic factors as tumour necrosis factor and interleukin-1. As a result of excessive lipid perioxidation endogenous DNA-reactive species are generated which again act as a driving force towards carcinogenesis. The last circumstantial evidence in this issue is that the frequency of mitochondrial deletions is highly encountered in skin neoplasia , a feature which is highly suggestive that oxidative stress if it is not the trigger of these neoplasias it participates in their development [36]

INFLAMMATORY SKIN DISORDERS

Free radicals are supposed to play a significant role in inflammatory skin diseases. ROS generation is responsible for follicular disruption and subsequent inflammatory changes in acne vulgaris. Psoriasis and Behcet disease are characterised by a predominant neutrophilic infiltrate which may be responsible for the generation of oxidative-induced inflammatory cascade [37-39].

Chemical burn

Chemical warfare agents were used first in the World War I in 1917 and the Iraq-Iran conflict in 1980s. HD or bis-2-chloroethyl sulphide induce skin cellular toxicity by oxidation and depletion of thiol groups including glutathione, which cause blister formation and necrosis [40]

Photodermatosis Melanin is the main chromophore in the epidermis absorbing photons with wave lengths from 350 to1200 nm, however, its protective effect is not highly competent. Unabsorbed photons by melanin access other atoms and molecules and the electromagnetic energy is transformed into chemical energy via two main oxygen atom dependent pathways. Type I pathway results in transfer of an electron from the excited triplet state into another molecule directly where reactive oxygen intermediates are formed . In type II pathway reacting atoms in the triplet state transfer energy directly to oxygen in the ground state producing a highl~fe2 ergetic singlet oxygen that oxidises a wide variety of biologic compounds [41,42] The pathogenesis of erythropoietic protoporphyria(EPP) and porphyria cutnea tarda (PCT) and possibly other types of porphyrias were thought for a long time to be mediated by reactive oxygen intermediates. Porphyrins have chemical similarities with chlorophill, the molecule can absorb photons from light as their absorbing spectrum is about 410 nm (visible light is between 400-700 nm). The excited molecules react with any organic compound in its vicinity. Both types of porphyrins EPP & PCT benefit from antioxidant therapy by carotenoids[43]

Vitiligo The pathogenesis of vitiligo is quite complex, one of the theories recognised is the autocytotoxicity theory. Electron micrograph examination of vitiliginous intertace and normal skin in patients showed accumulation of extracellular granular material and basal vacuolation of pigmented skin during disease progression with no lymphocytic infiltration in the vicinity which may be due to thioredoxin reductase inhibition with eventual increase in the oxidative stress. Reduced intracellular catalase level was also found in vitiliginous skin, with an extra oxidative burden to the melanocyte. In addition to the apoptotic effect on oxidative species, cell death may be facilitated by oxidation of dopamine to hydroxydopamine (ROI) which is quite lethal even to melanoma and neuroblastoma cell Iines [44,45]

INTERVENTION BY ANTIOXIDANTS

With the increasing incidence of skin cancer due to ozone depletion the desperate need for photoprotection became a major issue of interest to both scientists as well as lay people. Two years ago the American Academy of Dermatology award for young investigators was given to Wei H for his study on photoprotective effect of isoflavone genistein which reflects that the issue is highlighted by both investigators and supervisors [46]

ANTIOXIDANTS IN FOOD,DRUGS AND COSMETICS

At the beginning of this century, an entire language of specifically defined terms and acronyms that accompariied pharmaceutical development and regulation was settled in Europe (EMEA) or the European Medicine Evaluation Agency and in the USA(FDA)or the Food and Drug administration. The original food and drug act in 1906 was expanded in 1938 by the Food, Drugs and Cosmetic(FDC) act in 1938 prohibiting misbranding, adulterating, and requiring proof of safety and establishing drug regulation[47]. Antioxidants are used in food, drugs and cosmetics.

There is an increasing need to know more about these agents simply because of their spectral use in pharmaceutical products and their expanding consumers use or abuse. It is estimated that 70% of US population are using dietary supplementation which may be either prescribed or self administrated[48]

Topical Antioxidants

Alpha-glucosylrutin 1 % and Ferulic acid 0.05% were used separately in the prophylaxis against polymorphus light eruption with remarkable improvement. Ferulic acid and Caffeic acid are also used in combination to prevent UVB-induced erythema [49,50].

Aminophenol derivatives (o-aminphenol-4-methylpheno!) is a group of antioxidants and H1 antihistamines developed to bear anti-inflammatory effects as well. Several derivatives were tested on lab animal with proved efficacy in acute anaphylaxis, delayed hypersensitivity reactions and inhibition of lipid peroxidaton of rat brain homogenates [51]

Anthralin was used successfully in the treatment of psoriasis. Fifty nine simple analogues have been prepared by modifying the positions of 1 ,8 hydroxyl groups, replacement of the hydroxyl into functional groups at various positions in the anthracenone nucleus. They inhibit the perioxidation product leukotriene Ba by the neutrophils, though it is suggested by other authors that the efficacy of anthralin is due to its ROS generation[52]. Asiaticosides are derived from plant Cantella asiatica, 0.2% topical solution for one week in mice enhances the antioxidant enzymatic and non-enzymatic system of the skin [53]

Biomelanin and synthetic melanin showed a potent antioxidant effect comparable to that of vitamins C&E on lipid peroxidation, however, biomelanins exert more antioxidant effect than synthetic melanin. Augmentation of the antioxidant capabilities of biomelanins occurs on addition of ascorbate and tocopherols [54].

CoQ1O (ubiquinone) is decreased in ageing skin, when topically applied it decreases the depth of wrinkles via suppression of UVA-induced collagenase activation. The total level of oxidation is decreased as measured by weak photon emmision [55].

Glabridin is a major ingredient of hydrophobic fraction of licorice extract, topical solution of 0.5% inhibit UV-induced erythema and pigmentation via blocking of superoxide production [56].

Isoflavone genistein is derived from soybean, its anti-cancer effect is mainly due to its antioxidant activity through blocking UV-induced erythema, inhibiting lipid peroxidation and preventing DNA damage . It also blocks UVinduced DNA damage and modulates UVR-activated signal transduction, thereby suppressing the initiation and promotion of photocarcinogenesis. It also suppresses the protooncogenes c-fos and c-jun expression in mouse epidermis [46].

Lem 9 is a topical antioxidant derived from lemon oil tried both in-vitro and in-vivo. It increases the antioxidative potential of the biosuface [57]

Lipoic acid (5% alpha lipoic acid) decreases facial wrinkles without short term side effects [58]

Melatonin (N-acetyl-5-methoxytrytamine) was used topically alone or in combination with vitamin C and vitamin E before ultraviolet exposure the result was evaluated by non-invasive bioengineering methods and showed a dose dependent photoprotective effect of melatonin alone or incombination with vitamin C&E. The sun protective effect of melatonin is mainly due to scavenging of reactive oxygen species. Melatonin can be combined also with beta-carotenes in topical preparations [59,60].

Polypodium leucotomus an immunomodulator with antioxidant properties is effective both by oral and topical routes. It rises the minimal erythema dose (MED) and minimal phototoxic dose (MPD) in-vivo on human volunteers against UVA & UVB [61].

Procyanidin B5-3'-gallate is a polyphenolic fraction of grape seed. It is a powerful antioxidant. when used topically in a concentration of 0.5 to 1.5 mg/ mouse/ application it has an anti-tumour promoting activity. Epigallocatechin gallate is another gallate plant extract tried topically, extracted from green tea, it delays the onset on UV-induced skin cancer in mice [62,63],

Silymarin a flavinoid antioxidant isolated from milk, it prevents the oncogenic effect of cancer inducers(TPA and OA) in a dose dependent manner and if applied 6% topically before benzoyl peroxide, neutralises the neoplastic promotion effect [64]

Superoxide dismutase(Cu Zn SOD) is tried experimentally on murine and human skin with promising protective results against PUVA-induced inflammatory reactions [65]

Vitamin A has been used for decades topically in the treatment of a spectrum of indications and a diversity of mechanisms proposed among which is antioxidant capability [66].

Vitamin C (Ascorbate) is the newest topical preparation available for treatment of ageing .The use of topically applied vitamin C was delayed until a stable aqueous product with good skin penetration could be formulated. Vitamin C comprises equal amounts of the isomers L-ascorbic acid and D-ascorbic acid. Only L-ascorbic acid has the ability to be absorbed percutaneously. It is a water soluble antioxidant claimed to improve the

signs of ageing and photoageing, and used also in cancer prophylaxis. Cellex-C was the first commercially available topical vitamin C analogue. It is a 10% L-ascorbic acid in a stable, aqueous and acidic formulation. It is believed that zinc and tyrosine help to facilitate the absorption of vitamin C analogue in Cellex-C. The product penetrates directly into the dermis where collagen is synthesised. Topical vitaminC&E are combined with topical sun screens, however the efficacy is still unproven[67,68].

Vitamin D3 (1, 25 dihydroxy cholecalciferol) has a photoprotective effect attributed to metallothionin and inhibits UVB-induced erythema [69].

Vitamin E namely alpha-tocopherol protects the skin against oxidative UVinduced damage and reduces the formation of lipid perioxides with a total of 50% increase in reduced glutathione. In combination with vitamin C, it prevents the systemic UV-induced immunosuppression. Vitamin E alone or in combination with vitamin C and melatonin are ineffective in prevention of UVB-induced erythema when applied after exposure [60,70]

Systemic Antioxidants

Carotenoids (Provitamin A) are pigmented micronutrients present in vegetables and fruits, over 600 cartenoids including alpha-carotene, beta carotene, crocetin, canthaxanthin and fucoxanthin, however beta-carotene is the most widely studied Carotenoids function as accessory pigment in photosynthesis, and offer protection against photosensitization, via triplet excited carotenoid; and some carotenoids serve as provitamin A via central and eccentric cleavage. In addition to their antioxidant activity, carotenoids have an antimutagenic and antineoplastic effects [43,71] .

Carotenoids and vitamin A not only share structural similarities but also some mechanisms of action with marginal differences notably the antioxidant activity being higher in carotenoids. They are' used with well known efficacy in erythropoietic protoporphyria . The antioxidant effect is also claimed in cancer prophylaxis, their anticarcinogenic actions are multiple, including immunologic enhancement by increasing natural killer cells, upgrading connexin43 gene and inducing apoptosis. The anti-cancer effect of carotenoids is by far less than that of retinoids [72] .

Dapsone was used for along time in dermatologic practice, besides its antileprotic action it is an effective therapy in many neutrophilic dermatosis. The

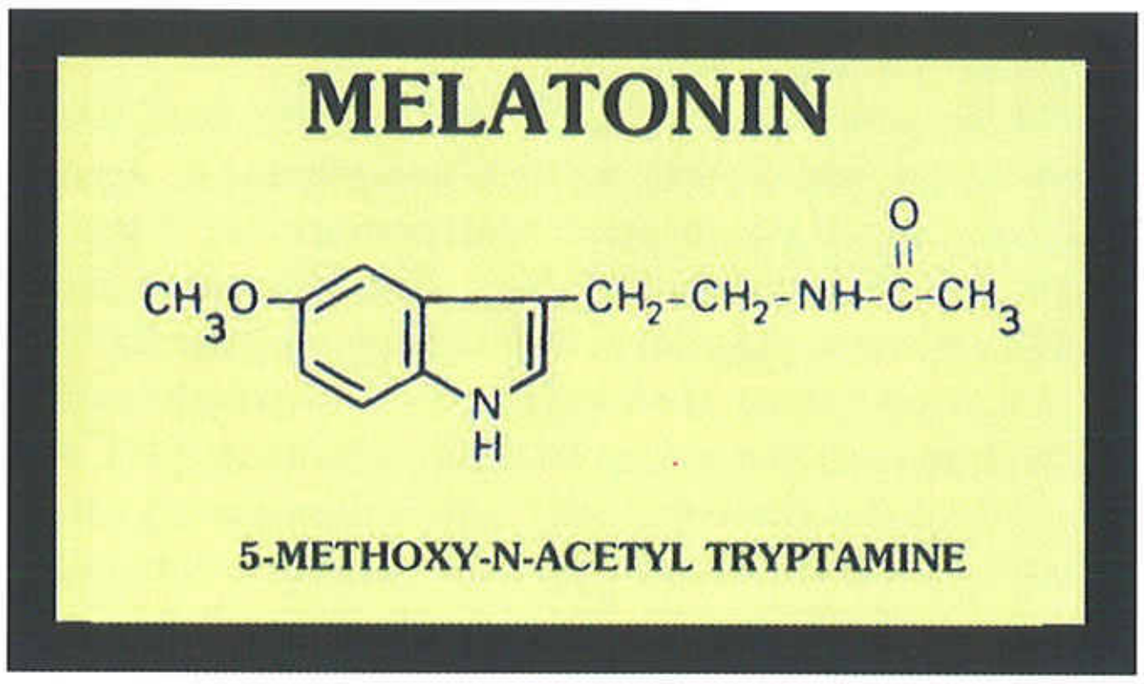

FIG 14 THE IDEAL ANTIOXIDANTS : MELATONIN

mechanism of action is by suppression of myeloperosidase, lipooxygenase and other oxidative mechanisms. Dapsone is an indirect antioxidant that reduces the overall production of reactive oxygen intermediates [73]

Melatonin is a potent endogenous free radical scavenger, hundreds of publications have confirmed clearly that melatonin is a broad spectrum antioxidant that quenches almost all nitrogen arid oxygen species. The advantage of melatonin over popular antioxidants is that it doesn't carry the possibility of a pro-oxidant activity, because it is not recycled. Instead melatonin acts as a suicidal antioxidant reacting with oxidative species forming a terminal and stable metabolite. It has an expanding list of indications among which is the claimed antiageing effects [74,75] (fig 14)

Selenium is an essential trace element has functions beyond being a component of the glutathione perioxidases. It is a constituent of iodothyronine deiodinase and thioredoxin reductase, which is a key enzyme in the redox state of the cells. Epidemiological studies showed that there is an inverse relationship between selenium and cancer incidence in the lung.colorectum and prostate with an overall 48% reduction in the mortality rate [72,76]

Vitamin A is defined generically to include all naturally occurring, nutritionally active forms of vitamin A as retinol and retinyl esters. Retinoids are synthetic derivatives or analogues of vitamin A and are mainly employed as drugs. A normal adult liver contains approximately 2 years requirement of vitamin A. It is the oldest well-characterised vitamin as mentioned by the Ebers papyrus that liver can treat eye diseases and it was also used by chinese physicians to cure night blindness. The chemical structure of vitamin A was

determined in 1930. One international unit is equivalent to 0.3 ugm of retinol. Antioxidant therapy with vitamin A in a dose of 25,000 IU/day has been given in oxidative stress caused by chronic alcoholism and smoking. The raised apprehension about the toxicity of vitamin A, and the paradox of having a prooxidant effect may limit its use as an antioxidant [77,78])

Ascorbic acid is essential for human life, the daily recommended dose varies from 40-60 mg/day, which is adequate by a wide margin to prevent scurvy. Increased daily requirements are recommended for smokers, cancer prevention and wound healing. It is a broad spectrum and standard antioxidant and a cofactor of a spectrum of physiological pathways in the body, in the skin it is an important contributor in collagen synthesis [77,79]

Vitamin E is a fat soluble vitamin. There is no organ of storage for vitamin E. One milligram is equivalent to one international unit, the US daily allowance is 30 mg/day. In a dose of 2 gm/day combined with 3 gmlday vitamin C, it prevents photocarcinogenesis via an antioxidants process. The mechanism of action is through the delay of UVA-induced apoptosis [77,80]

THE PARADOX: ANTIOXIDANTS AS PRO-OXIDANTS

It has been noticed during therapeutic trials that in the presence of the well known antioxidants, oxidative processes are going on without limitation. The antioxidants itself may be oxidised and oxidative intermediate is formed (ROI) particularly in the presence of transitional metals. This effect was demonstrated in-vitro for vitamin C. Also, the inclusion of uric acid in the incubation medium during copper-induced low density lipoprotein (LDL), oxidation exerted a pro-oxidant effect with tocopherol depletion. Other antioxidants as freulic acid may exert a pro-oxidant effect under special laboratory conditions. Vitamin E analogue Trolox, which has a water soluble molecule and lacks the phytyl chain may exert a prooxidant effect during metal-induced oxidation of (LDL)[81-84].

The pro-oxidant effect of vitamin C in the presence of iron has been used for decades to induce lipid peroxidation , oxidation of DNA and proteins, however, scientists argued that iron in the body is almost organically bound to proteins and the possibility of such pro-oxidant pathways is quite limited. Clinical studies provide a circumstantial evidence that during antioxidant therapy of ascrobate, tocopherols and beta carotene there is a significant increase in the level of excreted oxidative metabolites [85].(fig 15)

ASSESSMENT OF THE BODY OXIDATIVE STATES

It is essential with the growing belief that oxidation induce diseases per se, to assess the how much oxidation is going on inside. Qualitative and quantitative analyses were done, but still their application in clinical medicine still needs more refining. DNA oxidative metabolites are attracting more attention in researches because it is correlated with the mutagenesis and carcinogenesis. Eight hydroxy deoxyguanosine (8-hydroxy-2'-deoxyguanosine) is the oxidation product of guanine the most oxidizable DNA base. It was found in high concentration in the cancerous tissues and also in urine, so it is considered now the most useful indicator of DNA oxidation [86].

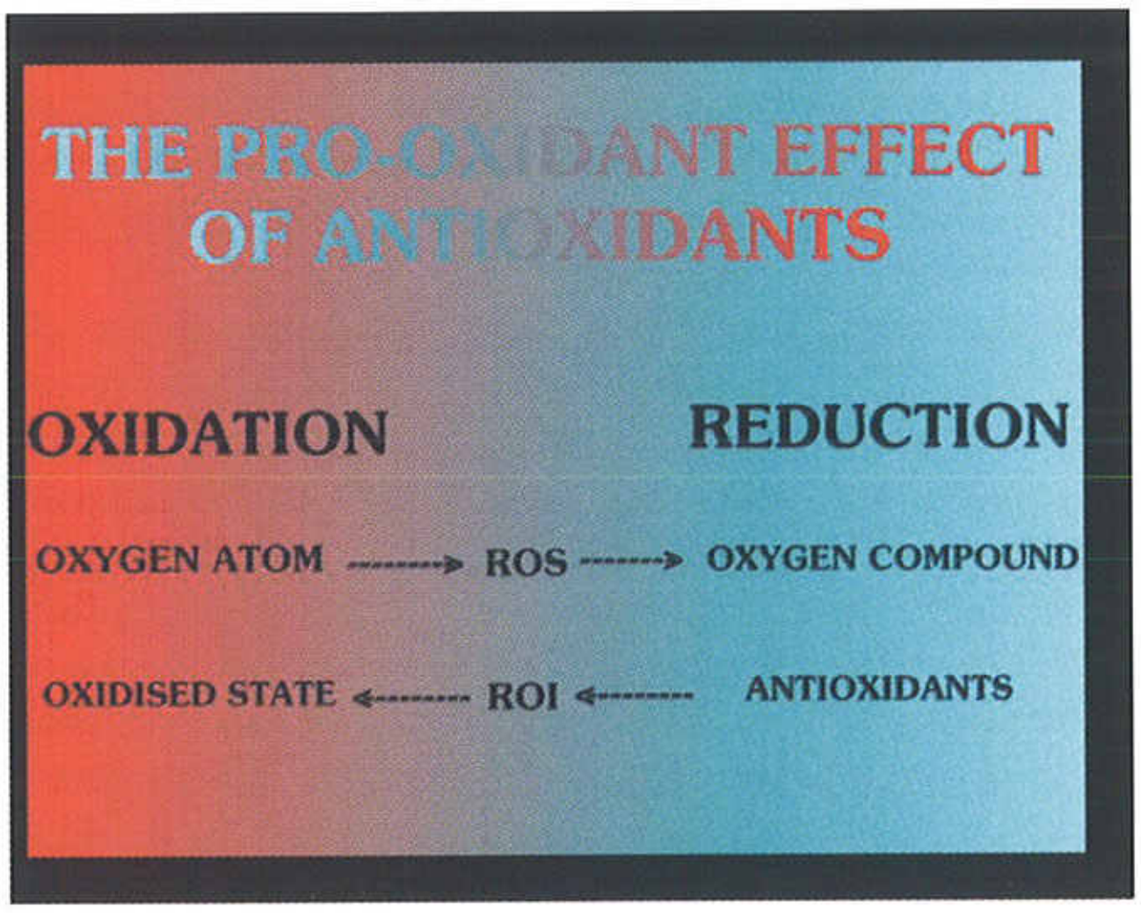

FIG 15 THE PRO-OXIDANT EFFECT OF ANTIOXIDANTS

Protein oxidation products are sensitive indicators of age-related degenerative changes, particularly the carbonyl derivatives. Other amino acid oxidative derivatives are: 2-oxohistidine (histidine), N-formylkynurenine (tryptophan), dihydroxy tyrosine (tyrosine), methionine sulphoxide (methionine). Lipid peroxidation products as thiobarbituric acid reactive substances (TBARS) are elevated in

septicaemic patients, however, malondialdehyde (MDA) the endproduct of oxidation of polyunsaturated fatty acids is the most frequently used biomarker of lipid peroxidation besides the other 22 saturated and unsaturated aldehyde products [87-89].

PRESPECTIVES OF ANTIOXIDANT USE IN DERMATOLOGY

Oxidative pathways are a part of all inflammatory processes occurring all over the body including the skin. Topics as neutrophilic and eosinophilic dermatoses might be a good target for future researches on the role of antioxidants. Besides the inflammatory disorders, the hot topics of ageing , photoageing and carcinogenesis are not well covered topics and will require more efforts to crystallise a clear concept about how much the oxidative processes are involved in their pathogenesis and if the current antioxidants or future antioxidants are effective in prevention or treatment.

CLOSING REMARKS

Skin is subjected to oxidative stress both endogenously and exogenously. The physiological antioxidant mechanisms of the skin are limited, so it is expected that antioxidants may play a considerable role in dermatology practice.

Antioxidants have been used in industry and medicine for decades, the question is why the subject is raised again and becomes a trend in many branches as cardiology; neurology, andrology, nephrology and at last dermatology. Is it the growing interest in molecular chemistry?(fig 16) Is it an impact of the great pharmaceutical companies to resale these items again under new indications?. Is it the pressure exerted by the "Naturalists" who are opposing and antagonising synthetic medicines? Or the result of the efforts of workers in the field of alternative or complementary medicine who convince patients that incurable diseases like cancers are managed by vitamins?! Is it the people's need or panic of photocarcinogenesis and photoageing indirectly encouraging researchers to find out a quick and simple remedy and this was propagated and inflated prematurely by media?

It is difficult to answer these questions or to put a clear cut opinion on what is going on, however, it is quite clear that antioxidants that carry great therapeutic expectations have so far a frank poor achievement.

FIG 16 WHY ANTIOXIDANTS BECAME A TREND IN MEDICAL SCIENCES

OXYGEN SPECIES

	Singlet	Superoxide	Peroxide	Hydroxyl
Symbol	$^1O_2$	O^-_2	H_2O_2	OH^-
Life Span	μ Sec	Short	10 Sec.	Ultra short
Existence	Abnormal	Normal	Normal	Normal
Reactivity	Low	Low	Low	High
Antioxidant Enzyme	-	SOD	PDase & Catalase	Hydroxylase

TABLE 1 COMPARING THE DIFFERNT OXYGEN SPECIES

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