ELEVATED 8-ISOPROSTANE LEVELS IN BASAL CELL CARCINOMA AND IN UVA IRRADIATED SKIN

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Isoprostanes are prostaglandin isomers produced from the peroxidation of polyunsaturated fatty acids from the cellular membrane. They have been used as a specific index of cellular lipoperoxidation and as an indirect measure of oxidative stress. However, these molecules also present several biological activities. An oxidative environment measured as the presence of other indirect measurements of reactive oxygen species lipoperoxidation has recently been described in basal cell carcinoma, the most frequent type of non-melanoma skin cancer. This study aims to measure the levels of 8-isoprostaglandin F2α, an isoprostane widely studied in other models as a by-product of ROS-induced lipid peroxidation, in basal cell carcinoma and in UVA irradiated healthy skin. We found that 8-iso-PGF2α is present in higher levels in BCC specimens compared to healthy non sun-exposed skin, confirming previous studies on the production of lipoperoxidation in this tumor. Moreover, we demonstrated that topical pre-treatment with a compound containing vitamin E is capable of reducing 8-iso-PGF2α formation in UV irradiated skin suggesting a role for isoprostanes in UV induced inflammation and eventually carcinogenesis and confirming the function of vitamin E as an antioxidant in this model.

F2 isoprostanes are a family of prostaglandin isomers produced by non-enzymatic free radical catalyzed peroxidation of polyunsaturated fatty acids from the cellular membrane. Due to their chemical stability isoprostanes can be seen as a sensitive and specific index of cellular lipoperoxidation in biological fluids and human tissues as shown by in *vitro* and in *vivo* studies.(1) Measurement of isoprostanes has been shown to be more reliable than other commonly used tests to measure the degree of oxatidative stress (2).

8-iso-Prostaglantin-F2α (8-isoPGF2α) is the most widely studied F2 isoprostane and, besides its role as an indirect index of lipid peroxidation, it presents several biological activities mediated through tromboxane A2 receptor (3-4).

Basal Cell Carcinoma (BCC) is a very common cutaneous cancer in Caucasians, locally invasive, with a low metastatic potential (5). Previous studies have demonstrated the presence of an "oxidative microenvironment" in BCC evaluated by different oxidative stress markers such as the thiobarbituric acid reactive substances (TBARS) a lipoperoxidation

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by-product index of ROS production (6). Lipid peroxidation could be an important step in the mechanism of ultraviolet (UV) rays induced carcinogenesis, through the production of lipid-DNA adducts that are genotoxic and mutagenic (7).

The aim of this study is to evaluate the level of 8-iso-PGF2α in BCC lesional skin comparing it to non sun-exposed healthy skin, sun exposed healthy skin and to UVA irradiated skin. Moreover, we investigated whether the mechanism of isoprostane production after UV irradiation could be prevented by topical application of α-tocopherol .

MATERIALS AND METHODS

Patients

Twelve patients (whose clinical characteristics are summarized in Table I) with clinical diagnosis of BCC were recruited from the Department of Dermatology at "G. D'Annunzio" University in Chieti, Italy. The study was approved by the local ethical committee. Skin excisions were performed after informed consent was given. In each patient, the tumor was excised and the centre of the lesion was processed for the study. In addition, biopsies were also collected from two other areas: the buttock (healthy non sun exposed skin) and from perilesional skin (healthy sun exposed skin). All skin samples were immediately incubated in 1ml Dulbecco's modified Eagle Medium supplemented with Bovine Serum Albumin for one hour in a Dubnoff shaking bath at 37°C. Finally, the medium was collected , frozen at -80 °C, liophilized and resuspended in distilled water (0.5 ml). 8-iso-PGF2α levels were measured by a radioimmunoassay (RIA) validated by an independent assay method (gas cromatography/mass spectrometry) (8). Data are expressed as pg of isoprostanes per mg of tissue.

To validate any difference in isoprostane levels between sun exposed healthy skin versus non-sun-exposed healthy skin and to assess the protective capacities of topically applied vitamin E, we recruited 5 healthy non-smoking, Caucasian volunteers (3 males, 2 females; mean age 50; range 35-60 years, skin type II or III). None of the participants had been exposed to excessive natural or artificial UV radiations in the 4 months prior to the recruitment. A fixed sub-erithrogenic dose of UVA (1 J/cm) was given in two distinct areas of 3cm² on buttock skin. Three days before UVA exposure, one of the two irradiated areas received a daily single topical applications of an α- tocopherol-acetate based thermophobic, low-residue foam vehicle (e-mousse™, Mipharm, Milan, Italy). The irradiation source was a bank of 6 Philips R-UVA fluorescent lamps (Philips, Milan, Italy) with an irradiance 6.5 mW/cm². After irradiation, skin punch biopsies (6 mm diameter) were taken from the irradied areas and from adiacent unexposed skin. Skin samples were incubated in Dubnoff shaking bath as described above to measure isoprostane production.

Statistical methods

Unpaired Student's t-test analisys was applied to find out any significative difference between the three specimen groups. $P < 0.05$ values were taken as significant.

RESULTS

In these studies we found detectable level of 8iso-PGF2α in all the samples analyzed. In particular, significantly high level of 8iso-PGF2α in tumor (mean 2.13 pg/mg, ± 1.3) and histologically healthy sun exposed peri-lesional skin (mean 1.69 pg/mg, ± 1.1) compared to non sun-exposed healthy buttock skin (mean 0.707 pg/ml, ± 0.3). (P<0.05). No significant difference was seen between tumor tissue and sun exposed perilesional skin (Fig.1). To validate our results we tested if the difference between non sun-exposed and sun exposed skin could be attributed to the effect of UV radiation on tissue oxidative stress generation.

We found detectable levels of 8iso-PGF2α in all the samples. Irradiated skin showed higher levels of 8-isoPGF 2α (mean 7.98 pg/ml) compared with un-irradiated skin demonstrating high levels of lipid peroxidation, on the other hand, vitamin E topically pre-treated irradiated skin showed lower levels (mean 1,23 pg/ml) of 8-iso-PGF2α, suggesting an anti lipid-peroxidation effect of α-tocopherol application in vivo (Fig. 2).

DISCUSSION

Reactive oxygen species (ROS) including singlet oxygen, hydroxyl radical, superoxide anion are a family of oxygen based free radicals normally present in the skin as the result of aerobic cellular metabolism or as a consequence of environmental or therapeutic agents action (UV radiation, photodynamic therapy, arsenic derivatives, microorganisms) (9-10). Although low levels of ROS have a physiological role in normal skin (11), higher
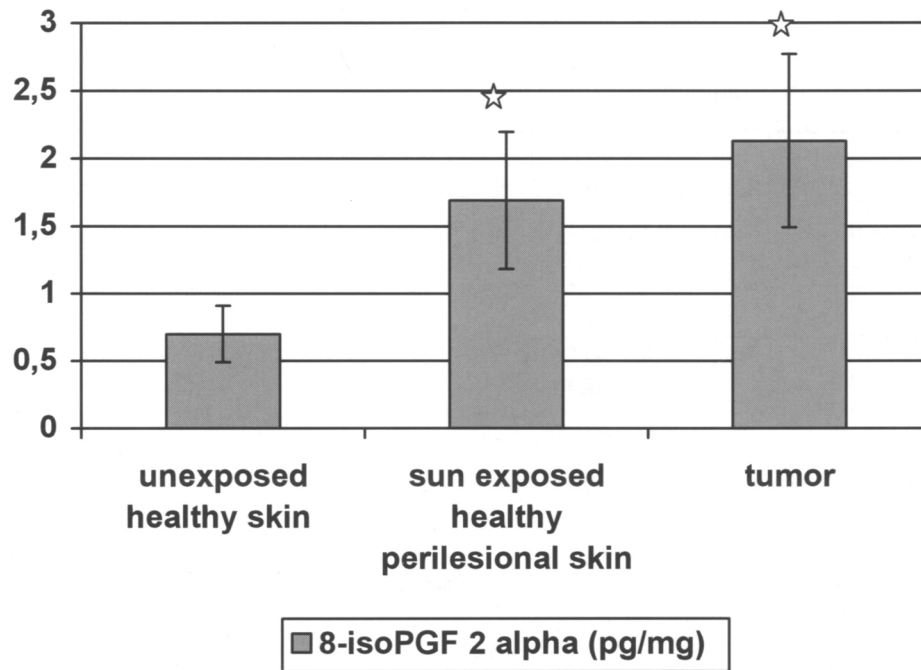
Fig. 1. *8-iso PGF 2α levels measured as pg/mg of tissue in lesional, sun exposed and unexposed skin of BCC patients (p<0.05 vs unexposed healthy skin).*

levels due to the inadequate removal of ROS by enzymatic and non-enzymatic antioxidant defense system, result in oxidative stress, with damage to several biological mechanisms and structures. Lipid membrane peroxidation is one of the effects of ROS removal failure, the by-products of lipid peroxidation may form adducts with DNA which are genotoxic or mutagenic(5) and lead to citotoxicity, cellular aging and carcinogenesis (12-13).

Basal cell carcinoma is the most common cutaneous cancer in Caucasian population. This tumor is locally invasive with a tendency to recurrence after surgery excision but rarely metastasizes. Studies have demonstrated a persistent oxidative microenvironment in cancer tissue including BCC (5,14). Higher levels of reactive biomolecules derived from ROS mediated lipid peroxidation such as TBARS, or DNA derived 8-hydroxi-2-deoxyguanosine (8OHdG), were found in BCC lesion compared to healthy controls (6).

The origin of the oxidative stimuli in BCC is unclear . Some studies have suggested that the BCC cells themselves are able to produce high amounts of ROS. BCC cells show an altered expression of different genes involved in the ROS defense system such as glutaredixin, glutathione S-transferase, superoxide dismutase, glutathione reductase, and glutathione oxidase, and catalases (15-16). Moreover, the presence of an inflammatory infiltrate in peripheral BCC tumor stroma with activated macrophages and lymphocytes may contribute to the oxidative condition (17).

The assessment of oxidative stress by the analysis of lipid peroxidation in human body fluids and tissues in pathological processes *in vivo* has been hampered by unreliability of assays. Diene conjugated and TBARS are characterized by a low sensitivity and specificity (2,18). Several studies have shown that quantification of 8iso-PGF2alfa in biological samples is a reliable, sensitive and specific method allowing the assessment of ROS-dependent lipid peroxidation *in vivo* (19).

To our knowledge this is the first time that 8iso-PGF2α is found in irradiated and tumors of skin. We show that 8-iso-PGF2α is generated in higher levels in BCC specimens compared to healthy non sun-exposed skin, confirming previous studies on the production of lipoperoxidation in this tumor.

To validate our results we tested if the difference between non sun-exposed and sun-exposed skin on oxidative level could be attributed to the effect of UV irradiation measuring 8-isoPGF2α production in
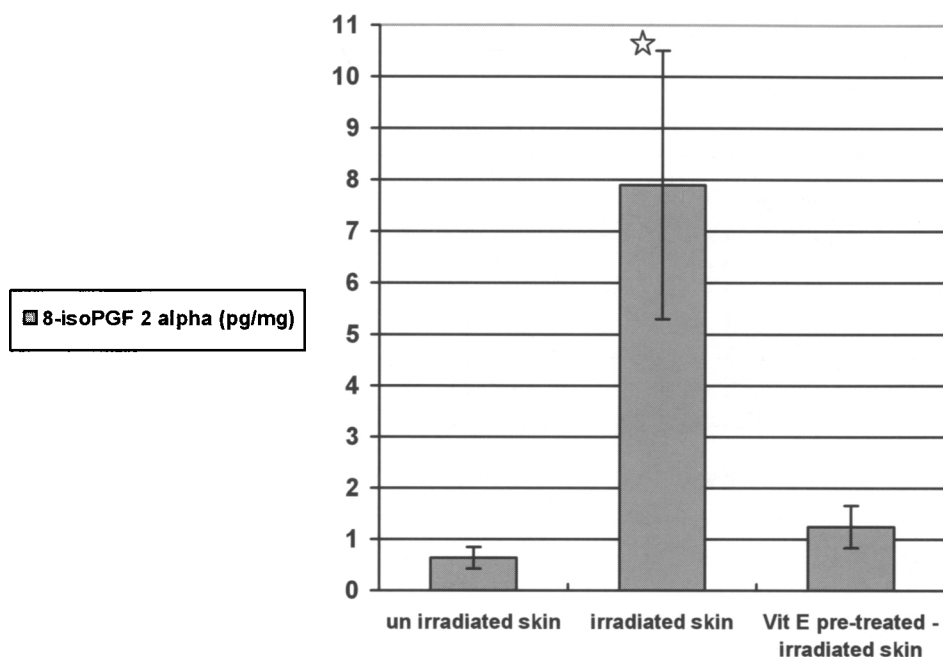
Fig. 2. *8-iso PGF 2α levels measured as pg/mg of tissue in UVA irradiated, unirradiated and Vit. E pre-treated UVA irradiated skin of healthy volunteers (p<0.05 vs unirradiated and Vit. E pretreated irradiated skin).*

UVA irradiated and unirradiated skin. We found higher levels of 8iso-PGF2α in UVA exposed healthy skin compared to the healthy non sun-exposed skin. Furthermore, we found that UVA induced 8iso-PGF2α production could be reduced by topical α-tocopherol pre-treatment.

Skin exposure to UV radiation is known to be responsible of several pathological conditions such as photoaging and photocarcinogenesis. These effects are mediated in part by UV induced oxidative stress through ROS production (20) coupled to the

impairment of tissue antioxidant defense systems (21). Several studies have indicated that topical applications of antioxidants like α-tocopherols have photoprotective effects on the epidermis in different models of UV photodamage, protecting cutaneous tissues from oxidative damage induced by UV irradiation *in vivo* (22-23). The high level of 8iso-PGF2α found in UV irradiated skin and in BCC could also have an intrinsic biologic activity 8iso-PGF2 is a well-known mitogen for endothelial cell (24). Moreover it modulates monocytes and

	12 Patients	5 Healthy Controls
Age years mean (range)	65 (50-85)	55 (40-60)
Sex F/M	5 / 7	2 / 3
Histological Tumor type		-
nodular	8	-
morfeiform	4	-
Phototype II/ III	6/6	2/3

Table I. *Clinical characteristics of patients and of healthy volunteers.*

neutrophil adhesion (25) and cytokine's production (26-27). We could speculate that the increased 8-iso-PGF2α levels may be correlated with the prominent neoangiogenetic process in BCC and with a potential role in tumor immunosurveillance.

Cyclooxygenase is an inflammatory enzyme responsible for the production of prostaglandins, that has been implicated in the development of UV induced epithelial cancers (28-30). Since isoprostanes could be produced even through this enzimatical pathway, it might be postulated a potential role for COX-2 derived 8-iso-PGF2a in cancer formation. Our future studies will focus on the inhibition of UV induced 8-iso-PGF2α formation in *in vitro* models by COX inhibitor drugs.

In conclusion, although it is still unknown if isoprostanes play any etio-pathogenetic role in BCC and UV photodamage, our findings of increased 8-iso-PGF2α levels support the involvement of ROS formation in these conditions and suggest a possible role of α-tocopherol antioxidant prophilaxis.

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