Effects of UV Radiation and Oxidative DNA Adduct 8-hydroxy-2′-deoxyguanosine on the Skin Diseases

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Excessive sun exposure may be associated with increased oxidative stress and oxidative damage of DNA. Based on this hypothesis, the authors focused on investigating the causal role of UV-induced genomic damage in the pathophysiology of porphyria cutanea tarda (photosensitive acquired metabolic disorder with autosomal dominant transmission). In this study we demonstrated that serum levels of 8-hydroxy-2′-deoxyguanosine, an altered nucleotide product, was correlated with body iron content (hemoglobin, sideremia, ferritin) in patients with porphyria cutanea tarda. Iron is involved in malignant processes by determining oxidative damage of DNA. Reduction of body iron content and genetic manipulation could have positive implications in prevention and progression of diseases mediated by oxidative stress.

Keywords: UV radiation, DNA adduct, 8-hydroxy-2′-deoxyguanosine (8-OHdG), iron store proteins, porphyria cutanea tarda

Excessive exposure to ultraviolet light (UV) is the main risk factor for skin cancer, sunburn, premature skin aging, some skin disorders associated with photosensitivity (porphyria, lupus erythematosus, vitiligo, rosacea, acne vulgaris), view disorders, decreased immune competence, worsening of pre-existing pathological conditions (collagenosis, psoriasis, cardiovascular diseases, rheumatoid arthritis) [1-5]. These findings were the base for specialists to investigate the role of genomic lesions induced by UV radiation in production of porphyria cutanea tarda (PCT).

UV spectrum of sunlight contains fields UV C (190-290nm), UVB (290-320nm), UVA (320-380nm). Stratospheric ozone retains full UVC radiations and partial UVB radiations. UVA and a fraction of UVB radiations reach the Earth’s surface.

UVB rays have a higher energy than UVA. UVB rays are absorbed directly by a number of cell components (nucleic acids, proteins, urocanic acid), exert mutagenic effects and are responsible for skin cancer development. UVA radiation acts on cellular constituents (nucleic acids, proteins, lipids, polysaccharides), exerting a weaker mutagenic effect than UVB.

UVA radiations operate on the body by oxidative mechanisms, correlated with reactive oxygen species formation. Typically, reactive oxygen species are grouped in:

- species with radical character: superoxide anion O₂⁻, hydroxyl radical HO, Nitrogen monoxide NO;
- nonradical species: oxygen singlet O₁, hydrogen peroxide H₂O₂, hypochlorous acid HOCl, lipid hydroperoxides.

UV photons are absorbed by the nucleic acids, proteins and various chromophores in the cell environment, such as quinones, steroids, porphyrins, flavin coenzymes, hemin proteins (cytochromes, peroxidase, catalase). The absorption of UVA photons by chromophores results in formation of reactive oxygen species or by transferring the energy to certain target molecules [5-12].

H₂O₂ is formed by UV irradiation of tryptophan. O₂⁻ anions are produced by UVA irradiation of NADH and NADPH. In the presence of iron ions, H₂O₂ and O₂⁻ can participate in Haber-Weiss reaction, and thus, resulting the production of hydroxyl radicals.

Fe²⁺ + H₂O₂ → Fe³⁺ + OH⁻ + HO /
Fe³⁺ + O₂⁻ → Fe²⁺ + O₂

Reactive oxygen species generated by UVA irradiation react with almost all molecules in biological systems: carbohydrates, phospholipids, nucleotides, organic acids, amino acids.

An important target of UVA are DNA macromolecules [1-4, 6, 8, 10, 11]. It is considered that UVA radiations interact with DNA by indirect mechanisms mediated by endogenous photosensitizers. UVA induces multiple types of DNA lesions, cleavage of phosphodiester bonds at the level of a single strand, the formation of DNA-protein cross-links, dimerization of adjacent pyrimidine (fig. 1). Cleavage of single-stranded phosphodiester bonds by cleavage of DNA is a major change during irradiation with UV rays comparing with photons UVB and UVC. Pyrimidine dimer formation is not a major change in DNA during irradiation with UVA, compared with irradiation with UV B and UVC.

UVA rays cause transcriptional activation of genes encoding heme oxygenase -1 (HO -1), metallothionein, CL100 phosphatase and collagenase [4, 9, 14]. Activation of HO-1 is a mechanism that controls the intracellular level of iron ions. High levels of iron ions promote lipid peroxidation dependent on iron. Heme oxygenase -1 and -2 are involved in heme and heme proteins degradation. Production of heme degradation, bilirubin, is a powerful antioxidant, while the compounds catalysed by heme oxygenase, porphyrins, act as photosensitizers in terms of UVA
irradiation, generating O$_2^\cdot$. In dermis and epidermis the mechanisms of constitutive (HO-2) and inducible (HO-1) protection are closely correlated, being modulated by intracellular levels of ferritin. HO-1 expression in response to the action of oxidant agents and to the decrease of reduced glutathione concentration in tissues not involved in hemoglobin degradation, suggest that heme degradation and heme proteins constitutes a protective mechanism against oxidative stress.

Metallothionein, activated by UVA radiation is involved in protection against oxidative stress by: scavenging of reactive oxygen species, superoxide radicals and series of organic radicals capture, ions binding Zn, Cd, Cu, blocking Fenton reaction by chelating iron ions.

CL100 gene encodes Tyr/Thrphosphatase that inactivates MAP kinase involved in the oxidative stress response.

Collagenase activation secondary to UVA irradiation represents a destructive response that causes degeneration of interstitial collagen phenomenon of fotosenescence..

In eukaryotic cells, there were identified pathways for repairing damaged DNA (fig. 1). In humans the studies proved the effectiveness for the next mechanisms for repairing genomic lesions: repair pathways, base excision repair (BER), nucleotide excision repair (NER), recombinational repair (homologous recombination/Non-homologous End-Joining-HR/NHEJ), mismatch repair (MMR), direct reversal - (methyl guanine methyl transferase, MGMT). Therefore, determination of oxidative stress markers in intact cells or tissues must be interpreted as a balance between level of aggression and rate of DNA repair at the time of obtaining the sample. One of the indicators used to assess the effect of oxidative stress on nucleic acids is represented by 8-hydroxy-2'-deoxyguanosine (fig. 2) [4, 8, 10]. Deciphering fundamental mechanisms of oxidation/DNA repair would allow identification of new therapeutic targets in diseases mediated by oxidative stress.
A photosensitive acquired metabolic disorder with autosomal dominant transmission is represented by porphyria cutanea tarda (PCT) [5, 11, 13, 15, 16].

Heme biosynthesis includes a sequence of reactions with well defined intermediate compounds: delta aminolevulinic acid (ALA), porphobilinogen (PBG), uroporphyrinogen III coproporphyrinogen III, protoporphyrinogen IX, protoporphyrin IX, heme (fig. 3) [5, 13].

These metabolic sequences are catalyzed by: coproporphyrinogen oxidase (EC number 1.3.3.3), protoporphyrinogen oxidase (EC number 1.3.3.4), oxygen-independent coproporphyrinogen-III oxidase (EC number 1.3.99.22), aminolevulinate synthase (EC number 2.3.1.37), porphobilinogen deaminase (EC number 2.5.1.61), uroporphyrinogen Decarboxylase (EC number 4.1.1.37), aminolevulinate dehydratase (EC number 4.2.1.24), uroporphyrinogen -III synthase (EC number 4.2.1.75), holocytochrome-c synthase (EC number 4.4.1.17), ferrochelatase (EC number 4.99.1.1), cytochrome c (EC number none).

Assuming that excessive sun exposure may be associated with oxidative degradation of DNA, the authors investigated the possible association between iron overload and serum levels of 8-hydroxy-2'-deoxiguanosine in porphyria cutanea tarda patients.

Experimental part

Materials and methods

Selection of patients. The authors excluded from the study patients with: alcohol poisoning, halogenated organic compounds or heavy metals, cirrhosis, fatty degeneration of liver, hematologic disorders, infectious diseases, metabolic syndrome, hemosiderosis, hereditary hyperbilirubinemia, malignancy, myocardial infarction, adverse drug reactions, hereditary tyrosinemia, acute porphyria, treatment with phenothiazines.

Inclusion criteria. 50 adult males diagnosed with naive porphyria cutanea tarda, (age = 56.8 ± 8.6 years, BMI = 22.1 ± 1.7 kg/m) and 50 healthy men (age = 53.6 ± 9.3 years, BMI = 23.1 ± 1.2 kg/m) were included in the study. The diagnosis of porphyric cutanea tarda was made by clinical examination and laboratory determinations.

Laboratory determinations:
- Full blood count was performed automatically with Pentra C60 (ABX, France).
- Determination of liver function tests, glucose, kidney tests, lipid profile and iron was made by photometric methods (HumaStar 300, GmbH, Germany).
- Ferritin, transferrin, and C-reactive protein were determined by immunoturbidimetric method (HumaStar 300, GmbH, Germany).
- 8-hydroxy-2’-deoxyguanosine (8OHdG) determination was done by ELISA method (TECAN, GmbH, Austria).
- Coproporphyrins and urinary uroporphyrins dosage was done by chromatographic and spectrophotometric methods (396-410nm).
- Aminolevulinic acid (ALA) and porphobilinogen in urine (PBG) determination were done by column chromatography with ion exchange resins and spectrophotometry (555nm).

**Biological samples**
- venous blood collected in anticoagulant vacutainer K3EDTA to determine haematological parameters;
- venous blood collected a jeun in vacutainer without anticoagulant with/without gel separator for serum determinations.
- urine collected within 24 h, stored at 2-8°C and kept in the dark to determine Coproporphyrins uroporphyrins, aminolevulinc acid and porphobilinogen

**Statistical analysis of data**
The clinical and laboratory quantitative data were expressed by mean and standard deviation. The correlation between the phenomena was expressed by the correlation coefficient r. The value threshold for the statistical significance was 0.05. The processing of data was performed using SPSS software.

The study was approved by the Hospital Committee of Ethics. All patients consented for the use of their biological samples in research and for teaching.

**Results and discussions**
PCT occurs especially in adult hood and affects usually males. PCT is triggered by liver disorders, resulting in elevated aminotransferase and gamma glutamyltransferase and low levels of butyrilcholinesterase (table 1).

The diagnosis of PCT was based on the following laboratory investigations:

- significantly increased serum levels of iron in PCT compared to controls (p<0.05);
- increased values of Coproporphyrins and urinary uroporphyrins in PCT compared to controls (p<0.05) (table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCT (n=50)</th>
<th>Control (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td></td>
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<tr>
<td>Hemoglobin (mg/dl)</td>
<td>17.9±2.7**</td>
<td>14.1±2.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>58.1±8.1**</td>
<td>41.5±1.4</td>
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<tr>
<td>Serum</td>
<td></td>
<td></td>
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<tr>
<td>AST (U/L)</td>
<td>87.3±22.1**</td>
<td>21.3±5.2</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>79.4±19.3**</td>
<td>18.4±6.1</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>69.4±8.3**</td>
<td>19.2±4.3</td>
</tr>
<tr>
<td>BChE (U/L)</td>
<td>3411±876**</td>
<td>5912±702</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.4±0.5**</td>
<td>4.1±0.2</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>1.1±0.9**</td>
<td>0.19±0.19</td>
</tr>
<tr>
<td>Iron (ug/dl)</td>
<td>204±61**</td>
<td>117±38</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>186±59**</td>
<td>274±86</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>304±109**</td>
<td>64±48</td>
</tr>
<tr>
<td>Uroporphyrins in urine (ng/ml)</td>
<td>34.2±1.1**</td>
<td>5.1±3.8</td>
</tr>
</tbody>
</table>

**Urine**
- Coproporphyrins (ug/24h) | 466±202** | 18±18 |
- Uroporphyrins (ug/24h) | 735±311** | 4.3±4.3 |
- ALA (mg/24h) | 4.8±2.3 | 0.2±0.2 |
- PBG (mg/24h) | 1.2±1.2 | 0.4±0.4 |

1 - p < 0.05 statistically significant variation between phorpyria cutanea tarda versus control
n - number of patients, PCT = phorpyria cutanea tarda, AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = gamma-glutamyltransferase, BChE = Butyrylcholine esterase, CRP = C-reactive protein, 8-OHDG = 8-hydroxy-2’-deoxyguanosine, ALA = Aminolevulinic acid, PBG = Porphobilinogen

- increased concentration of hemoglobin in PCT patients compared to control (p<0.05);
- significantly increased serum levels of ferritin in PCT compared to controls (p<0.05) (table 1).

- Level of aminolevulinic acid (ALA) and porphobilinogen (PBG) in urine collected in 24 h showed no statistically significant differences between patients with PCT and control (p>0.05) (table 1). These biochemical abnormalities recorded in patients with PCT were caused by a moderate reduction of the uroporphirinogen decarboxylase [8, 11, 13, 15, 16].

Iron overload evidenced in patients with PCT was confirmed in this study also by other results (table 1):
- increased concentration of hemoglobin in PCT patients compared to control (p<0.05);
- significantly increased serum levels of ferritin in PCT compared to control (p<0.05);
- significantly lower serum of transferrin in PCT compared to controls (p<0.05).

Under physiological conditions most of body iron is found in compounds with heme [5, 8, 11, 15, 16]. Non hemin iron is stored as ferritin or hemosiderin in macrophages and hepatocyte. A very small amount of circulating iron is bound to transferrin. Alteration of hepcidin regulating mechanism (protein with negative effects on serum iron) or of signaling molecules involved inregulation of hepcidin synthesis plays an important role.
in pathogenesis of diseases caused by disorder of iron metabolism.

Cutaneous manifestations in patients with PCT increased after exposure to sunlight. This finding is justified experimentally by obtaining significantly higher serum concentrations of 8OHdG in PCT patients compared to controls (fig. 4).

Another important aspect obtained in this study is the significant positive correlation, registered in PCT patients between serum levels of 8OHdG and hemoglobin concentration (fig. 5), between serum levels of 8OHdG and sideremia (fig. 6), between serum levels of 8OHdG and ferritin (fig. 7). A negative relationship with statistical significance was obtained between serum levels of 8OHdG and transferrin in patients with PCT (fig. 8). In control group, total body iron content showed no association with serum levels of 8OHdG. In PCT patients, the association between total body iron content and serum levels of 8OHdG is even stronger.

### Table 2

<table>
<thead>
<tr>
<th>Study group</th>
<th>Variable</th>
<th>Hemoglobin (ng/mL)</th>
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<tbody>
<tr>
<td></td>
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<td>&lt; 16</td>
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<tr>
<td>PCT</td>
<td>8OHdG</td>
<td>23.1±3.4</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td>8OHdG</td>
<td>5.0±3.6</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.63</td>
</tr>
</tbody>
</table>

n= number of patients; 8OHdG=8-hydroxy-2'-deoxyguanosine; PCT=porphyrin cutanea tarda; r=correlation index; p=statistical significance

### Table 3

<table>
<thead>
<tr>
<th>Study group</th>
<th>Variable</th>
<th>Ferritin (ng/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤ 200</td>
</tr>
<tr>
<td>PCT</td>
<td>8OHdG</td>
<td>35.5±12.2</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td>8OHdG</td>
<td>5.4±2.3</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.04</td>
</tr>
</tbody>
</table>

n= number of patients; 8OHdG=8-hydroxy-2'-deoxyguanosine; PCT=porphyrin cutanea tarda; r=correlation index; p=statistical significance
stronger when metabolic abnormalities are more pronounced (tables 2-5).

Although it is accepted that inactivation of uroporphirinogen decarboxylase is the main pathophysiological cause of PCT, the results obtained in this study supported the involvement of oxidative DNA modifications in disease pathogenesis. These results could be explained, in the author conception, in that patients with iron overload could promote inflammation, oxidative stress, mitochondrial dysfunction, impaired synthesis of hepcidin. As previously reported, the authors assume that oxidative DNA lesions associated with cellular redox imbalance, could be central element for the development of hepatocellular carcinoma in patients with PCT [8]. Production of oxidative lesions in DNA caused by reactive oxygen species, in relation with genetic and nongenetic factors, could induce an exacerbation of metabolic deficits, resulting in a decrease in uroporphirinogen conversion in coproporphirinogen.

Conclusions

This study demonstrated that 8OhdG-a modified gene product was correlated with altered iron stores in the body and hence with severity of PCT. Iron accelerates the process of malignant transformation by DNA damage-dependent oxidative reactions.

The association between iron overload, oxidative DNA adduct could be regarded as a new risk factor in a number of diseases mediated by oxidative stress. PCT patients with increased risk for developing hepatocellular carcinoma can be identified by increased serum levels of 8OhdG and ferritin.

Therapeutic methods of decreasing the iron content in organism (therapeutic phlebotomy, iron chelators use), diet, genetic manipulations, reducing sun exposure could have a decisive role in reducing the risk of carcinogenesis.

References

1. KATO M, IWASHITA T, TAKEDA K, AKHAND AA, LIU W, YOSHIIHARA M, YAJIMA I, Sunlight Exposure-Mediated DNA Damage in Young Adults, Cancer epidemiology 2011;20,1622
7. NICOLAE, C, NICOLAE, CEASUS E, THEOCE, F, GEORGESCUL S R, Rev. Chim. (Bucharest), 64, no. 6, 2013, p. 654

Fig. 7. Graphical representation of 8-hydroxy-2-deoxyguanosine (8-OhdG-ng/ml) in relation with to ferritin(nm/ml) in patients with porphyria cutanea tarda and control group (p <0.05)

Fig. 8. Graphical representation of 8-hydroxy-2-deoxyguanosine (8-OhdG-ng/ml) in relation with to transferin(nm/ml) patients with porphyria cutanea tarda and control group (p <0.05)

F, Oxidative DNA damage correlates with cell immortalization and mir-92 expression in hepatocellular carcinoma, BMC Cancer2012, 12(1):177
7. NICOLAE, C, NICOLAE, CEASUS E, THEOCE, F, GEORGESCUL S R, Rev. Chim. (Bucharest), 64, no. 6, 2013, p. 654

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