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CANCER BIOLOGY

**OXIDATIVE STRESS IN CANCER PRONE XERODERMA PIGMENTOSUM
FIBROBLASTS. REAL-TIME AND SINGLE CELL MONITORING OF SUPEROXIDE
AND NITRIC OXIDE PRODUCTION WITH MICROELECTRODES**

**Stéphane ARBAULT¹, Neso SOJIC¹, Delphine BRUCE¹, Christian AMATORE¹,
Alain SARASIN² and Monique VUILLAUME^{1,2}.**

¹ *Département de Chimie, UMR CNRS 8640 « PASTEUR », Ecole Normale Supérieure
24 rue Lhomond, 75231 PARIS Cedex 05. FRANCE*

² *Institut Gustave Roussy, PR2, UPR CNRS 2169, 39 rue Camille Desmoulins, 94805 VILLEJUIF.
FRANCE*

*Present address for N. Sojic and D. Bruce: LACReM, ENSCPB, 16, Avenue Pey-Berland,
33607 PESSAC. FRANCE*

Corresponding author: Dr. Stéphane ARBAULT. Département de Chimie, UMR CNRS 8640 « PASTEUR », Ecole Normale Supérieure, 24 rue Lhomond, 75231 PARIS Cedex 05. FRANCE
E-mail: Stephane.Arbault@ens.fr / Tel: 33.1.44.32.36.41 / Fax: 33.1.44.32.24.02

ABSTRACT

Sun exposure is clearly implicated in premature skin ageing and neoplastic development. These features are exacerbated in patients with *Xeroderma Pigmentosum* (XP), a hereditary disease associated at the cellular level with DNA-repair defects and a low catalase activity. The implications of oxidative stress in the defects and cancer proneness of XP skin cells (keratinocytes, fibroblasts) are multiple and remain unclear. They were investigated here at the level of a single fibroblast by an electrochemical method based on microelectrodes we have previously developed. These microelectrodes permit a real-time quantification and identification of superoxide and nitric oxide derivatives (H_2O_2 , ONOO^\cdot , NO^\cdot , NO_2^\cdot) released by a living cell following its stimulation. Then, the oxidative bursts produced by fibroblasts from normal strains were compared to those of fibroblasts from several XP group A (XPA) and XP group D (XPD) strains. All XPA and XPD strains provided responses of higher amplitude and duration than controls. The XP specific oxidative response could not be correlated with DNA repair ability since the transduction of XPD strains with the wild type *XPD* gene did not modify their production of reactive oxygen and nitrogen species. The nature of these species was investigated and revealed that cancer prone XPD fibroblasts produced higher amounts of $\text{O}_2^{\cdot-}$ and H_2O_2 and lower amounts of NO^\cdot and than normal fibroblasts.

INTRODUCTION

There are strong evidences that oxidative stress is a central issue in processes of ageing, transformation or death of living cells and thus leads to many pathological processes among which the induction of cancers [1]. Oxidative stress basically originates from an acute or chronic imbalance between the production of reactive oxygen (ROS: H_2O_2 , OH^\bullet , $^1\text{O}_2$, ...) and nitrogen species (RNS: NO^\bullet , ONOO^\bullet , NO_2^\bullet , ...) and antioxidant capacities of living cells and organisms. Among all the cellular targets of ROS and RNS (membranes, proteins, enzymes), damaged DNA is repaired by base excision repair and nucleotide excision repair systems (NER) [2], which finally safeguard the integrity of the genetic material in an error-free manner. Deficiencies in NER give rise to some human genetic diseases, *i.e.*, Xeroderma Pigmentosum (XP), Cockayne's syndrome (CS) and Trichothiodystrophy (TTD), according to different mutations on one of the DNA repair genes [3]. Seven different NER genes, *XPA* to *XPG*, defining the XPA to XPG genetic groups, are involved in the XP disease which principal hallmarks are a high predisposition (x 2000 as compared to the general population) to developing epidermal skin tumors (melanomas for 20 % of patients, basal cell and squamous cell carcinoma for almost all patients) onto day light-exposed body area and neurological abnormalities for some complementation groups [4], [5], [6].

We previously established that cell strains of fibroblasts and keratinocytes from XP skin biopsies present an abnormal low catalase activity compared to control cell strains [7], [8]. This biochemical dysfunction of the cellular defense against ROS was correlated with the NER defect in XP because the transduction of XP diploid cells by the wild type *XPD* or *XPC* gene fully corrected NER defects and restored catalase activity to the level of controls [9]. Low catalase activity of XP skin cells could not be attributed to differences in the level of catalase transcription or amounts of catalase protein subunits but to other metabolic disorders as for instance, low NADPH cellular levels [8]. These disorders might be the consequence of unrepaired mutations (Figure 1) possibly induced and amplified by oxidative stress.

The key role of oxidative stress in the development or amplification of the metabolic impairments of XP cells was studied with an electrochemical method developed by some of us [10], [11]. The principle of this method is to place a microelectrode within micrometers of an isolated single living cell and monitor in real-time the release of electroactive ROS and RNS following the cell stimulation (Figure 2). We established that oxidative bursts were easily stimulated by a physical stress on the cell membrane through its puncture by a micropipette and the ensuing local depolarization [12]. Oxidative bursts occur within a short time scale and consist of a complex cocktail of several important metabolic effectors which nature and fluxes of emission could be determined by amperometry at different electrode oxidation potentials: hydrogen peroxide (H_2O_2), peroxynitrite (ONOO^\bullet) and nitrogen monoxide (NO^\bullet), as well as nitrite ions (NO_2^-) as a result of partial peroxynitrite decomposition. For normal fibroblasts, all these species result from the ultimate combination of equimolar primary production of superoxide ion ($\text{O}_2^{\bullet -}$) and nitrogen monoxide (NO^\bullet) generated by two distinct enzymatic systems: respectively, NADPH-oxidase type enzymes for $\text{O}_2^{\bullet -}$, and NO-synthases for NO^\bullet , as revealed by the use of classical inhibitors [13].

We wish to present here the investigations by this electrochemical method of the nature, intensity and kinetics of oxidative stress of XP fibroblast strains in comparison with DNA-repair proficient strains. We have shown that XP fibroblasts from two complementation groups, XPD and XPA, chosen as references because of their very high NER deficiencies and low catalase activity, exhibit significantly larger oxidative bursts than control cells. The composition of ROS and RNS in the cell responses revealed striking differences for H_2O_2 and ONOO^\bullet releases between XPD and controls. Finally, the amplified oxidative stress response of XPD fibroblasts could not be modified after recombinant retrovirus complementation by the wild type gene *XPD*, which led us to believe that this property is not basically related to the NER pathway. It probably shows unrevealed metabolic impairments of the XP fibroblasts that may be important in the process of UV-induced

skin carcinogenesis.

MATERIAL AND METHODS

Cell strains. Wild type diploid human fibroblast strains were used as controls [CTR: 198VI (I); 405VI (II) and C5RO (III)] throughout this work and compared with the diploid fibroblasts strains from patients Xeroderma Pigmentosum of the group D [XPD: XP22VI (IV); XP26VI (V) and XP1BR (VI)] and Xeroderma Pigmentosum of the group A [XPA: XP4LO (VII) and XP18BR (VIII)]. Cell strains which name following the number is VI (for Villejuif, France) were established by some of us (UPR CNRS 2169) from unexposed skin biopsies and the other ones were obtained from other laboratories. The *in vitro* population doubling number of the cells used in these experiments was comprised between 7 and 18. The complementation of XPD fibroblast strains (XPD + D) number IV and V was obtained through the use of the recombinant retroviral vectors LXPDSN, which mediated the transfer and the expression of the wild type *XPD* cDNA, and allowed the G418-selection of genetically modified cells [14]. Cells were transduced and selected as already described [14]. UV sensitivity and unscheduled DNA synthesis (UDS) tests were used to evaluate DNA repair ability of all cell strains [15].

Cell culture and preparation for the oxidative stress measurements. All fibroblast strains were grown in MEM medium (Gibco BRL) with fetal calf serum (10%) in an incubator (5% CO₂, 37°C). Confluent monolayers of cells were harvested by trypsination. 1000 to 2000 cells were then re-suspended in each Petri dish (3.5 cm diameter, Costar 3035) and analyzed 48 hr later. Cells were washed three times with PBS just before the experiments performed in PBS at room temperature. To correct from differences in the data usually observed from one day to another, each XP strain response was tested in parallel with a control strain under the same experimental conditions.

Stimulation of a cell oxidative stress response by a physical stress. Experiments were performed on the stage of an inverted microscope (Axiovert 135, Zeiss, Germany) where was positioned the Petri dish with cells to study. Displacements of the detecting microelectrode (see below) and of a sealed glass microcapillary (1mm glass rods, GR100-10, Clark Instruments, GB; pulled with a PB7 Puller, Narishige, Japan) were controlled with two micromanipulators (MHW-103, Narishige, Japan). Under optical control, the microelectrode tip (sensitive area at its extremity) was precisely placed at a distance of $5 \pm 1 \mu\text{m}$ above the surface of an isolated living fibroblast while the tip of the microcapillary (*ca.* $1 \mu\text{m}$ radius) was positioned between the microelectrode surface and the cell (figure 2). Then, the microcapillary was moved rapidly via the z axis knob of the micromanipulator in order to puncture the cell membrane and taken back to its initial position in less than 1 s. This was possible under manual control because of the only few microns distance necessary to displace the microcapillary. This physical stress and ensuing local membrane depolarization activated enzymatic systems producing the oxidative bursts [11], [12]. Our cell stress protocol that resembles a microinjection protocol induced a cell oxidative response in more than 95 % of experiments.

Electrochemical measurements of a single cell oxidative stress response. Platinized carbon fiber microelectrodes (10 μm diameter, electrical charge for platinum electrodeposition ranging from 80 to 90 μC) were prepared according to the procedure reported previously [10], [12]. We have demonstrated their interest as highly sensitive probes for several superoxide and nitric oxide derivatives [12]. Each extracellular oxidative burst induced by physical stress was composed of ROS and RNS quantitatively collected by the microelectrode and analyzed by amperometry at constant potential *E* vs. SSCE (Sodium Saturated Calomel Electrode, the reference electrode). The detection method offered a sensitivity in the range of sub-femtomoles and a time-response better than 100 ms. Kinetics of ROS and RNS releases induced peak-shaped signals (Figure 3a) individually characterized by their maximum current (*I_p* in picoamperes), their electrical charge (*Q_p* in picocoulombs) and their

half-width ($t_{1/2}$ in seconds). Mostly, the electrical charge was used to evaluate the magnitude of the cell response since this parameter is directly proportional to the number of molecules detected while I_p and $t_{1/2}$ are related to the kinetics of release. The potential of detection was set at +560 mV vs. SSCE for the experiments aimed to compare different cell strains or patient groups, since it offered the higher sensitivity for main ROS and RNS (excluding nitrite) [10]. Some experiments were also conducted at +300, +450, +650 and +850 mV vs. SSCE to determine the chemical composition of oxidative bursts on XPD cells in comparison with controls [12], [13], [16]. The three higher potentials corresponded to the plateau currents of the electrochemical oxidation waves (maximum sensitivity) detected with platinized carbon fiber microelectrodes on pure solutions of the following compounds: NO° , ONOO^- , H_2O_2 , and NO_2^- . Measurements at +300 mV were used to analyze the composition in the response of H_2O_2 / ONOO^- , two species oxidized at near positive potentials [13], [16]. The currents obtained by subtracting peaks detected at two successive plateau potentials were converted to fluxes for each compound based on the Faraday's law [12] and the reaction scheme in Figure 5. According to this scheme, we were able to estimate the fluxes of production of the two primary species, $\text{O}_2^{\circ-}$ and NO° , originating from the activation of NADPH oxidase and constitutive NO synthase [13].

RESULTS

Comparison of the oxidative stress response of control, XPD and XPA fibroblasts.

A specific oxidative stress response of human wild type fibroblasts was previously established [10], [11]. It was characterized in real-time and at a single cell level by amperometry at microelectrodes, which provided peak-shaped signals as a function of the time-course of cellular releases of ROS and RNS (see Figure 3a). In this study, we observed first that the amplitude and kinetics of amperometric peaks did not vary significantly, within the limits of the experimental reproducibility (*i.e.* $\pm 10\%$ for the minimum set of data), between three different control strains of fibroblasts (see Figure 3b), previously characterized for their DNA repair ability and catalase activity [8]. Based on that constant behavior of controls strains, we have compared their response in independent experiments to that of several fibroblast strains originating from XPD and XPA patients (Figure 3 b). For a better understanding of the results, the mean peak amplitude detected on each strain (control, XPD or XPA) was normalized *versus* a common reference, *i.e.*, the peak amplitude obtained for the normal strain most often used in our studies (named I: 198VI).

Larger responses were detected on all XP strains in comparison with all control strains, showing that XPD and XPA fibroblasts were recurrently able to produce higher amounts of ROS in our experiments. For each XP strain, the difference of peak amplitude *versus* the one of controls was always statistically significant although some noticeable variations among the five different XP strains were observed (see strain V vs. strain VI in Figure 3b). Raw average of the results obtained with all XP strains (XPD+XPA) led us to estimate that these fibroblasts generally yield responses 60% larger than normal ones. We observed that these differences of amplitude always correlated with differences of kinetics of ROS cellular releases, as evidenced by the changes of peak's shape in Figure 3a. The responses of XPA and XPD fibroblasts were wider (higher $t_{1/2}$) and lasted for longer times which means that these cells produced ROS more intensely over longer periods and therefore led to higher total quantities of species than controls. The significant and reproducible difference of response observed for the two groups of XP cells (XPD and XPA) in comparison with several controls implied that a specific oxidative behavior of these fibroblasts was linked to their XP genotype.

Oxidative stress response of XPD fibroblasts after transduction by the wild type *XPD* gene.

We have previously shown that the retroviral-mediated transduction of XPD fibroblasts by a wild type *XPD* cDNA restored to the level of normal fibroblasts many of the XPD cells defects, such as DNA repair ability and catalase activities [14], [9]. In order to investigate the relationship between NER deficiency and the oxidative stress activity of XPD fibroblasts, the responses of two XPD cell strains were analyzed before and after transduction by a wild type *XPD* cDNA. In both strains, the genetic correction of the cells did not significantly modify their responses. The amplitude of peaks

detected on corrected cells (see Figure 3b) was very similar to that of XPD cells (decreases of about 5 %) and no changes on the peak shape may even be noticed meaning that no difference of ROS and RNS production kinetics was detected. These results clearly established that the genetic correction of XPD fibroblasts by wild type *XPD* cDNA could not decrease their level of oxidative stress to the one of normal fibroblasts (statistically significant difference) and that this response was uncoupled to the *XPD* gene activity though it could be related to the XP origin of the cells (*vide infra*).

Chemical composition of the oxidative stress response of control and XPD fibroblasts.

Since XP fibroblasts (XPD and XPA groups) displayed specific oxidative stress responses and secreted more ROS and RNS than control fibroblasts, the identification of the species produced by the different type of cells became a central point of our studies. This was achieved following a methodology we have recently reported [13], [16] to study the chemical composition of ROS and RNS and quantify their fluxes in the oxidative bursts of human normal skin fibroblasts. This method was based on amperometric measurements at several oxidation potentials (see material and methods) determined to offer a high selectivity for each compound detected in the cell response. Then, based on the reaction scheme in Figure 5, the fluxes of production of the two primary species, *i.e.*, NO° and $\text{O}_2^{\circ-}$, which lead to the complex cocktail of species composing the extracellular bursts, were determined.

XPD fibroblasts were then analyzed following the protocol established on normal fibroblasts. XPD cells did not release any significant flux of ONOO^- while the amounts of its decomposition product, NO_2^- , were approximately two times smaller than for controls (Figures 4a, 4b). The amounts of NO° freely diffusing in the medium were equivalent for both cell types (Figure 4b) while larger quantities of H_2O_2 were produced by XPD relative to controls (3.2 times, Figure 4a). Estimated fluxes of the original production of superoxide and nitric oxide differed also significantly between cell types: XPD cells yielded a 40 % increase of $\text{O}_2^{\circ-}$ and 30 % decrease of NO° production than controls (table in Figure 4). These differences were correlated with changes in the production rate of $\text{O}_2^{\circ-}$ between XPD and controls (Figures 4d, 4e) while the kinetics of NO° production was similar for both cell types (Figures 4c, 4e). Despite that the initial flux of superoxide, *viz.*, during the first seconds following the cell activation, was not significantly different between cell types, XPD cells finally produced $\text{O}_2^{\circ-}$ over longer times than controls (compare the slower decay of the flux versus time for XPD on normalized curves in Figure 4e) and thus led to a larger cumulative production of this species.

DISCUSSION

The quantitative results presented above were obtained owing to the electrochemical method we previously established [10], [11]. The analyses were based on the measurements by a microelectrode of the releases of reactive oxygen and nitrogen species by a single living cell following its activation. We have shown before on different cell types that the insertion of a microcapillary's tip into a cell was sufficient to induce the release of ROS and RNS' bursts [10], [11]. This physical stress on the cell membrane may be compared to the stress induced by solid particles (e.g., asbestos particles) or other mechanical stress as shear flow, which have been both shown to induce oxidative bursts [17], [18]. The physical stress by a glass microcapillary on single human fibroblasts and leucocytes (lymphocytes, macrophages, neutrophils) activates very rapid, unique and intense oxidative bursts, which are function of the metabolic capacities to produce rapidly ROS and RNS, and may be related to cellular responses involved in inflammation processes [10], [19]. Oxidative bursts could be on the one hand, diminished by specific enzymatic inhibitors of NADPH oxidases or NO synthases and on the other hand, amplified through the effect of Tat or gp160 proteins of HIV-1 on lymphocytes [19] or through the effect of a PMA pre-activation on rodent macrophages (unpublished results). Then, our experimental setup gives the possibility to measure rapidly, *i.e.*, in the range of a few minutes, the ability of a single cell to produce ROS and RNS and to investigate the implications of this oxidative stress in pathological situations.

Through this approach, we studied here the relations between ROS and RNS production and the development of skin cancers based on the model of fibroblasts from *Xeroderma Pigmentosum* patients who display a high predisposition to developing epidermal skin tumors. We have tested in comparison with fibroblasts from human skin controls the oxidative response of XP fibroblasts for two complementation groups, XPD and XPA, which are known to lead to some of the most severe physiological deficiencies in XP patients. We observed a sensitive, reproducible and significant increase of ROS production for both groups. Then, our results clearly established the existence of a relationship between the XP origin of the cells and their high oxidative stress response.

The possible relationship between the XP specific response and the deficient DNA repair ability was studied in the case of cells mutated on the *XPD* gene since we could test two strains of XPD fibroblasts before and after complementation by a normal copy of the *XPD* gene [14]. Complemented cells exhibited an oxidative stress activity equal to that of non-complemented ones although their responses to other tests (UDS, kinetics of death after UV irradiation, catalase activity) was equivalent to that of normal cells and not that of XPD [9]. Thus, this suggests that the DNA repair deficiency state of XP cells at the time of experiments is not correlated with their activity involved in intense oxidative bursts, which could rather be due to an epigenetic state of XP cells [21]. Moreover, since XP fibroblasts are able to produce high levels of ROS in addition with their characteristic low catalase and glutathion peroxidase activities, this situation might favour the amplification of DNA damages in these cells. Although DNA damages produced by ROS might be removed primarily by the base-excision-repair pathway (BER), which is efficient in XP cells, it was recently reported by Kuroaka I. *et al.* [20] that a subclass of base lesions in DNA exposed to ROS (purine cyclodeoxynucleoside adduct) could not be removed by the BER. These lesions could even be partially repaired by NER enzymes. It was concluded that this form of DNA damage independently of the DNA lesions induced by UV might be responsible for defects in XP individuals. Then, These and our results favour the hypothesis that the specific high level of oxidative stress in XPD and XPA cells may be independent of *XPD* and *XPA* genes but might be partially responsible for XP pathology and carcinogenesis.

Furthermore, our electroanalytical method gave the possibility of analyzing the nature of the different species contributing to the specific oxidative stress response of XP fibroblasts. This work was conducted on XPD cells for the reasons described above. We observed a larger production of $O_2^{\bullet-}$ and a lower production of NO^{\bullet} by XPD fibroblasts than in controls. This situation led to the releases of lower amounts of $ONOO^{\bullet-}$, even when considering also the contribution of its decomposition compound, $NO_2^{\bullet-}$. In correlation, larger amounts of H_2O_2 were released while the quantity of unreacted NO^{\bullet} , *i.e.*, that released by the cells in the extracellular fluid, was unchanged. When considering the additional effects of oxidants versus the ones of UV on XP cells, larger amounts of H_2O_2 may be harmful for the cells since it is well known that this species is quite stable, relatively to the above mentioned species, and may then potentially reach all cellular compartments to generate hydroxyl radicals OH^{\bullet} , which high reactivity towards DNA is well known. This situation should favor the formation of a sub-class of DNA damages (purine cyclodeoxynucleosides) that can not be removed by the BER, as reported by Kuraoka I. *et al.* [20]. These lesions could even be partially repaired by NER enzymes. In this context, the high level of oxidative stress in XPD and XPA cells may be independent of *XPD* and *XPA* genes but might be partially responsible for XP pathology and carcinogenesis.

Large amounts of hydrogen peroxide were released by XPD fibroblasts because of a sustained production of superoxide as compared to controls. The kinetics of superoxide and nitric oxide productions significantly differed on XPD (slower decay of $O_2^{\bullet-}$ than of NO^{\bullet}) while they were equivalent on controls. These results suggest an increased activity of superoxide generating-NADPH oxidases in XPD cells, as recently proposed for skin fibroblasts from patients with systemic sclerosis [22]. Several hypotheses may explain our observations: (i) more enzymatic sites releasing $O_2^{\bullet-}$ may exist on the XPD cell membrane (NADPH oxidases are trans-membranic enzymes); (ii) an increasing recruitment or larger amounts of enzyme subunits (p47, p67 or p22_{phox}) will lead to a higher synthesis of $O_2^{\bullet-}$. It was demonstrated that NADPH oxidase assemblies (membranic gp91_{phox} with cytosolic subunits) to become active [23] and that a continuous replacement of the subunits is

necessary for a long term generating capacity [24]. It was also recently reported that the activation of osteoclasts by IFN- γ increased NADPH oxidases components and superoxide production [25] or that macrophage activation by an antibiotic enhanced mobilization of the subunits and releases of $O_2^{\bullet-}$ [26]; (iii) a different use of the metabolic pools by the NADPH oxidases in XPD and normal cells. Superoxide and nitric oxide generating systems may also compete for the use of the cofactor NADPH or for ATP [8]. We observed before that a constant proportion between ROS exist for cells from the same strain or group despite the cell variability [12]. This led us to consider the possible existence of regulating mechanisms coupling the two enzymatic systems, NADPH oxidase and constitutive NO synthase, during the whole oxidative burst so that they perform in phase. In this context, it was reported by several groups that the activity of NADPH oxidase was modulated (increase or decrease) by nitric oxide or peroxynitrite as a function of their concentration range [27], [28]. Those mechanisms could result different in XP fibroblasts leading to more efficient superoxide-generating systems.

Moreover, a change of the ratio between H_2O_2 and $ONOO^-$, the two follow-up products of superoxide, was detected on XPD cells. The kinetics of release of H_2O_2 is directly related to that of $O_2^{\bullet-}$ while that of $ONOO^-$ is a function of both kinetics of $O_2^{\bullet-}$ and NO^\bullet releases. Since we observed a faster decrease of NO^\bullet production than the one of $O_2^{\bullet-}$ on XPD, $ONOO^-$ kinetics follows NO^\bullet kinetics and peroxynitrite is then probably generated for shorter times and then in lower quantities than H_2O_2 . However, this may not be sufficient to account for the observed 50 % decrease of $ONOO^-$ (quantity of NO_2^- comprised) so we believe that other conditions govern the production of peroxynitrite [16]. For instance, inducible NO synthases (iNOs) may be involved in XPD in conjunction or in replacement of constitutive NOs (cNOs) to produce NO^\bullet . We have recently observed (unpublished results) that the induction of iNOs in macrophages by LPS + IFN- γ decreased the amounts of $ONOO^-$ released. We believe that the location of NO^\bullet generating systems may vary and that NO^\bullet may thus take longer time to reach $O_2^{\bullet-}$ generating sites [16]. This would lead to lower quantities of peroxynitrite because of the competitive disproportionation of $O_2^{\bullet-}$ into H_2O_2 versus its reaction with NO^\bullet . This competition is obviously also dependent on the local concentration of SOD and on the local pH where the reactions take place. All these hypotheses need to be tested in the future by investigating the specific conditions of NADPH oxidases and NO synthases activities in the XP cells at the molecular level.

In summary, this work has demonstrated the unique capacity of the fibroblasts from XP patients and furthermore, from the XPD group, to produce larger amounts of ROS and in particular H_2O_2 , a potent mutagenic compound, in addition with the low activity of their antioxidant defenses such as catalase. This property is not directly linked to the DNA repair capacity of the cells but correlates with their XP origin. These results may open new directions for understanding the role of oxidative stress processes in the XP pathological mechanisms among which, the neurological defects and skin neoplasms.

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LEGENDS TO FIGURES

Figure 1.

Genetic map of the ERCC2/XPD gene showing the positions of the mutations known in the cells from our DNA repair-deficient patients. The two alleles for each patient are indicated by the superscripts 1 and 2. The unscheduled DNA synthesis (UDS) values are indicated in parenthesis in % of wild type value 4 hours after 15 J/m² UVC as measured in our laboratory. The UDS value for XP1BR was reported to be about 30 % [7]. The boxes indicate DNA/DNA and DNA/RNA helicase domains for the XPD protein containing 761 amino acids.

Figure 2.

Optical microscopic view and schematic side-view (scale not respected) showing the principle of the electrochemical detection of oxidative stress bursts released by single human fibroblasts. The microelectrode (black shadow on right) and the micropipette (white shadow on left) used to trigger the oxidative stress response are placed with micromanipulators with respect to the cell adherent to the surface of the Petri dish.

Figure 3.

Comparison of the oxidative stress response of normal (CTR), Xeroderma Pigmentosum (XP) from the group D (XPD) and from the group A (XPA) human fibroblasts. (a) Typical amperometric peaks detected for XPD, XPA and controls fibroblasts. The black arrow represents the moment of activation of the cellular response by the microcapillary. Each curve is the average of individual cell responses from the following strains: CTR: strain I, XPD: strain V, XPA: strain X. (b) Comparison of the amplitude (total electrical charge) of amperometric peaks detected on the following strains: CTR = 198VI (I), 405VI (II) and C5RO (III); XPD = XP22VI (IV), XP26VI (V) and XP1BR (VI); XPA = XP4LO (VII) and XP18BR (VIII); XPD+D = XP22VI (IV) and XP26VI (V) after retroviral-mediated complementation by the *XPD* (+D) cDNA. Each value was obtained from the mean of 100 to 250 individual cell responses (each experiment was repeated 2 to 4 times to get representative data) and was calculated as a ratio to the mean value for the control strain 198VI in the same experimental conditions. Significant differences versus controls (*t* test 95% confidence) are indicated with an asterisk.

Figure 4.

Comparison of the individual fluxes of species released in the extracellular fluid (a): H₂O₂ and ONOO⁻; (b): NO₂⁻ and NO^o; or effectively produced (c): NO^o_{prod.} and (d): O₂^o_{prod.}; by normal control and XPD fibroblasts during oxidative bursts. The total quantities of NO^o_{prod.} and O₂^o_{prod.} detected on control and XPD fibroblasts by integrating curves in (c) and (d) are compared in the table. (e) Kinetic profiles of nitric oxide and superoxide productions by control and XPD cells, compared by normalizing the corresponding curves in (c) and (d) to their maximum current intensity. Each curve in (a-e) on the Figure represents the average of at least 30 individual cell responses.

Figure 5.

Reaction scheme describing the origin and the formation of the species detected in the oxidative stress bursts. The percentages of H₂O₂, ONOO⁻, NO₂⁻ and NO^o simultaneously detected in the oxidative burst are indicated in bold face for XPD fibroblasts and compared with the values in parenthesis for controls.

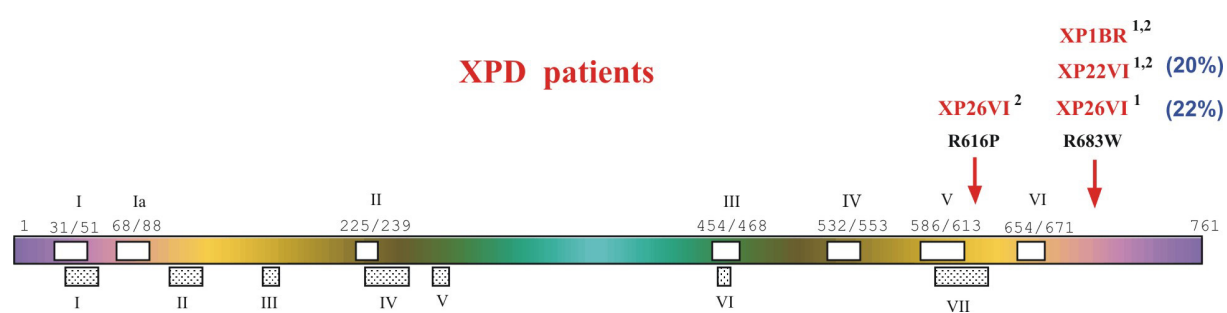
FIGURE 1

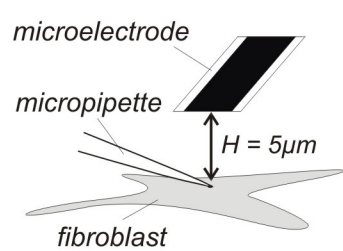
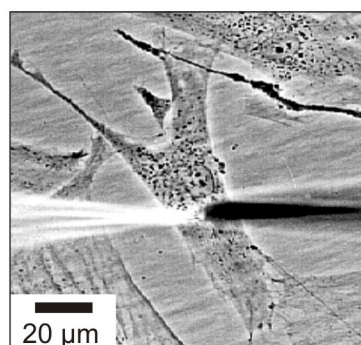
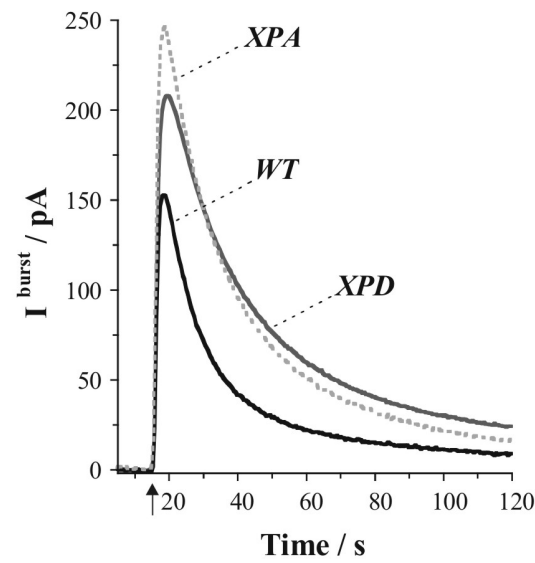
FIGURE 2

FIGURE 3

a)



b)

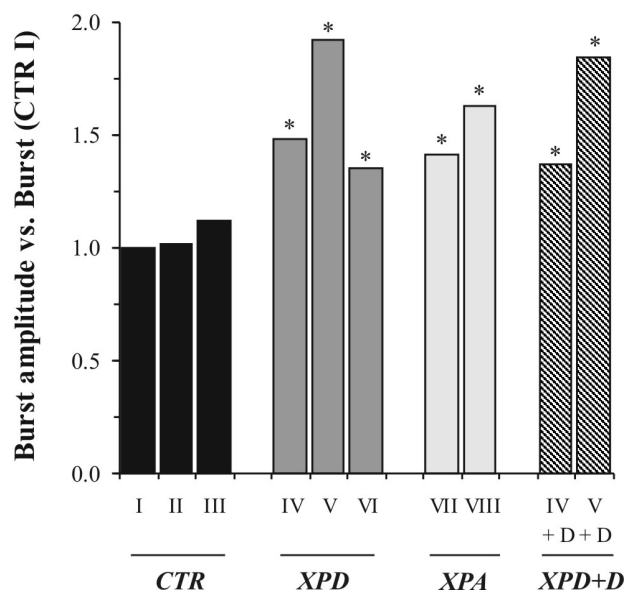


FIGURE 4

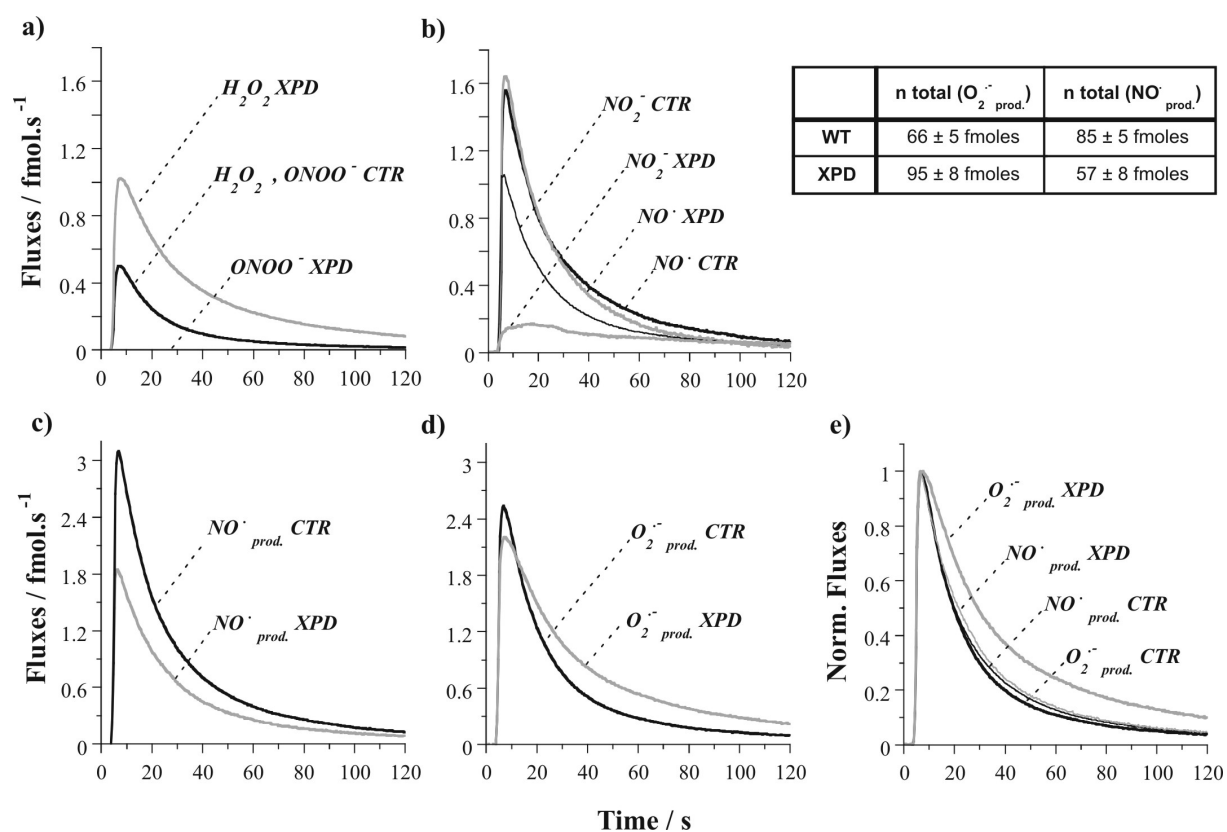


FIGURE 5

