ORIGINAL ARTICLE

Bleomycin and Olaparib Induced Modulation of Poly (ADP-Ribose) Polymerase 1 Activity and Chromatin Structure in Thymocyte Nuclei

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Abstract

Objective: Poly (ADP-ribose) polymerase 1 inhibitors (PARPi) are used in the treatment of cancer patients as monotherapy agents or in combination with approved anticancer drugs to optimize their clinical use. However, the development of novel anticancer chemotherapy is tempered by the potential detrimental side effects displayed by cytotoxic drugs in healthy cells. To delineate the rationality of PARPi olaparib (OLA) in combination with the approved DNA-damaging anticancer drug bleomycin (BLM), we investigated the influence of the drugs on poly (ADP-ribose) polymerase 1 (PARP 1) activity, chromatin, and DNA structure in healthy rat thymocyte nuclei. **Materials and Methods:** Wistar albino male rats (Rattus norvegicus, 6 weeks old) were used throughout the experiments. Animals were obtained from the stock of the animal house of the faculty of biology at YSU. The enzymatic assay for PARP 1 activity was performed according to the original method based on the estimation of residual NAD^+ concentration in the PARP assay mix adapted by us to quantify NAD^+ consumed by isolated nuclei. DNA concentrations were measured by the spectrophotometric method using an extinction coefficient ε_{260}=6600 M^{-1} cm^{-1}. Statistical differences in results between groups were evaluated by the Student's *t*-test. $P < 0.05$ was considered significant. **Key Results:** It was observed that in healthy rat thymocyte nuclei, BLM induced PARP-1 inhibition and DNA and chromatin loosening. In contrast, treatment with OLA maintained chromatin and DNA condensation, which could partly underlie the cytotoxic effect of OLA as a result of drug-induced down-regulation of chromatin-associated nuclear functions. Our findings provide evidence for the revalidation of the rationality of OLA application in combination chemotherapy regimens involving BLM.

Key words: Bleomycin, chromatin condensation, DNA melting, nuclei digestion, olaparib, PARP

INTRODUCTION

Poly (ADP-ribosyl) ation (PARylation) is a well-defined posttranslational protein modification accomplished by the members of the poly (ADP-polymerase) enzyme family (PARPs), also named the ADP-ribosyl-transferase diphtheria toxin-like enzyme family. This evolutionary-conserved family of proteins is involved in a broad spectrum of biological activities, e.g., DNA repair, cell death, division, and differentiation. Seventeen PARPs have been described up to now, and all of them utilize NAD^+ as a substrate to produce nicotinamide (Nam) and the ADP-ribose moiety. The vast majority of the members of the PARP family function as mono ADP-ribosyl–transferases, realizing mono (ADP-ribosyl) ation, which is ADP-ribose moiety transfer and linkage to glutamate, lysine, arginine, or serine residues of target proteins. The other members of the family, PARP 1, 2, 3, 5a, and 5b, are capable of sequential "protein-distal" addition of ADP-ribose moieties through the formation of ribose-ribose bonds between ADP-ribose units, thus carrying on the elongation of ADP-ribose chains. This reaction is recognized

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as poly ADP-ribosylation (PARylation) and is responsible for the anchoring of negatively charged ADP-ribose polymers (PARp) on target proteins. The length of PARp can reach up to 100 nm, and the chain can form branched structures.[1] The most profound and well-recognized member of the PARP family is PARP1, which is localized to cell nuclei. PARP 1 has well-defined structural domains that determine the different activities of the enzyme. The prominent feature that segregates PARP 1 from the other members of the family is self PARylation of the enzyme. During this process, PAR strands bind to amino acid residues within the autopoly ADP-ribosilation domain of PARP 1, located in the central region of the molecule between residues 374 and 525.[2] To a lesser extent, PARP 1 accomplishes trans-PARylation of other acceptor proteins. PARP 1 has a key role in DNA damage detection and in triggering DNA repair at the site of the lesion. The activity of PARP 1 is stimulated more than 500-fold upon binding to DNA breaks.[3] In addition to chromatin-associated proteins, PARP 1 can modify the free ends of DNA at the site of the DNA break.[4,5] PARP 1 is involved not only in DNA damage recognition and repair but is recruited to DNA in non-regular DNA structures (e.g., non-B-form DNA, crusiforms, and hairpin structures) during normal physiological processes such as transcription and chromatin remodeling.[6,7]

Poly (ADP-ribose) polymerase 1 (PARP 1) activation pathways play a significant role in the coordination of metabolic events with chromatin-associated processes involved in DNA repair, replication, and transcription. Considering the role of PARP 1 in DNA repair, enzyme inhibitors were approved for cancer treatment to potentiate the effect of DNA-damaging agents by hindering DNA repair. The accumulation of DNA lesions in cancer cells leads to replication collapse, mitotic collapse, and eventually cell death. However, PARP-1 inhibitors lack high enzyme specificity and are recognized in general as PARP inhibitors (PARPi). Since 2009, PARP have been employed as monotherapy agents for the treatment of BRCA-mutated breast and ovarian cancer or in combination with DNA-damaging agents in different chemotherapy regimens.[8] PARPi olaparib (OLA), rucaparib, niraparib, and talazoparib have been approved by the U.S. Food and Drug Administration and by the European Medicines Agency and will enter the clinic.

It was demonstrated that OLA inhibits PARylation and that PARP 1 is "trapped" onto the chromatin, which drives cytotoxicity in cancer and healthy cells.[9]

One of the powerful DNA-damaging agents widely used in anti-cancer treatment is bleomycin (BLM). It is a metallo-glycopeptide antibiotic employed in anticancer treatment either as a monotherapy agent or in combination with other anti-cancer agents. BLM induces DNA breaks by virtue of free radical-dependent processes, which seize replication and transcription and eventually lead to cell death. In addition, the cytotoxic effect of BLM could be improved by inducing apoptosis via the release of cytochrome C from mitochondria.[9]

Since, in combination with OLA, BLM induces cytotoxicity in healthy cells of the bone marrow,[10] the therapeutic advantage of PARP inhibitors in combination with host BLM treatment needs further examination. In the present study, we address the question of whether treatment of healthy rats with BLA could affect PARP 1 activity in thymocyte nuclei and influence the inhibitory potential of OLA. We were also interested in studying whether the PARP 1 trapping activity of OLA could affect thymocyte nuclei, chromatin structure, and DNA melting profiles.

MATERIALS AND METHODS

Animals

The experimental protocols and procedures were approved by the National Centre of Bioethics (Armenia) and the European Community regulations on animal experimentation for scientific purposes (D.M. 116192; O.J. of E.C. L358/1 12/18/1986) and performed according to the International Recommendations (CIOMS, 1985) guidelines. Ethical Committee of the Yerevan State University, Armenia.

Wistar albino male rats (Rattus norvegicus, 6 weeks old) were used throughout the experiments. Animals were obtained from the stock of the animal house of the faculty of biology at YSU. Rats were housed in laboratory conditions (20 ± 2°C) with a light/dark cycle, fed commercial rat feed ad libitum, and given free access to water. Animals were standardized by weight (100 g) and divided randomly into three different groups. Control group animals were injected intra-peritoneally (i.p.) with saline; injected i.p. with BLM resolved in saline (15 mg/kg); and injected i.p. with OLA (15 mg/kg) dissolved in DMSO followed by dilution in saline before injection. In 24 and 48 h after drag administration to animals, rats were killed under light ether anesthesia by decapitation.

The thymus gland was collected and accurately squeezed through cheesecloth to separate thymocytes from the epidermal and fibrous tissues of the gland. Nuclei were isolated, according to Hewish and Burgoyne.[11] Sucrose solutions utilized throughout the nuclei isolation procedure were buffered with 20 mM Tris containing 15 mM NaCl, 60 mM KCl, 0.15 mM spermine, and 0.5 mM spermidine, pH 7.4.

Naked nuclei were incubated for 2 h in isolation media. BLM, OLA, ATP, $CaCl_2$, and $MgCl_2$ were added to the nuclei's incubation media when needed. At the end of the incubation time, nuclei were sedimented (700 g, 10 min) and suspended either in PARP assay buffer or in lysis solution for further DNA isolation and electrophoresis according to protocols.[12]

PARP 1 assay

The enzymatic assay for PARP 1 activity was performed according to the original method based on the estimation of residual NAD^+ concentration in the PARP assay mix, adapted by us to quantify NAD^+ consumed by isolated nuclei.[13] Briefly, nuclei were gently suspended in PARP assay buffer containing 20 mM Tris, 6 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4. The density of the nuclear suspension was normalized to 1 mg DNA/mL. The PARP reaction was initiated by the addition of NAD^+ stock solution to a 1000 µL aliquot of nuclear suspension (0.5 mM NAD^+ final concentration). The reaction was carried out for 10 min at 37^0C and quenched by the elimination of nuclei from the reaction mixture. Nuclei were abstracted from the reaction mixture through centrifugation (13000 g, 4°C, 2 min). The nuclear pellet was discarded, and 50 µL aliquots samples of supernatant were transferred to a Falcon UV-Vis transparent 96-well plate. NAD^+ quantitation was performed by the sequential addition of 2M KOH, acetophenone (20% in EtOH), and 88% formic acid, in accordance with the original assay. The absorbance of the PARP assay mix containing 0.5 mM NAD^+ was measured at 378 nm. The amount of NAD^+ was determined by using the NAD^+ calibration curve, and PARP 1 activity was defined as NAD^+ consumed by nuclei in 10 min per mg of DNA.

All reagents were purchased from Sigma-Aldrich. BLM was purchased from MCE (US).

DNA fragmentation assay

100 µL aliquot samples of nuclear suspension normalized to 1000 µg/mL DNA were transferred to the Eppendorf tubes, and 60 mM $MgCl_2$ and 10 mM $CaCl_2$ were added to yield working concentrations of 6 mM $MgCl_2$ and 1 mM $CaCl_2$ in aliquot probes. The ions were added to activate endogenous Mg^{+2}- and Ca^{+2}/Mg^{+2}-dependent nuclear endonucleases, which initiated internucleosomal DNA cleavage.[14] Nuclear DNA was subjected to electrophoresis on 1.8% agarose gels (8 v/cm). DNA was visualized by ethidium bromide staining.

DNA melting

DNA concentrations were measured by the spectrophotometric method using an extinction coefficient of ε_{260}=6600 $M^{-1}cm^{-1}$. The melting of DNA isolated from naked thymocyte nuclei was performed on a UV/VIS PYE Unicam-SP8-100 (UK) at the maximum wavelength of 260 nm. The heating of a thermostat-controlled cell (3 mL) was carried out employing the SP 876 Series 2 program device. The length of the optic pathway was 1 cm. The heating rate of the thermostat-controlled cell was 0.5°C/min, and the temperature was automatically recorded every 60 s. Data emerged on a PC monitor employing LabView software. Temperature and absorption data were modified and saved with the Microsoft Excel Office 10 software package.

Reagents

BLM and OLA were purchased from MCE (US). All reagents used throughout cell nuclei preparation, DNA isolation, and processing were purchased from Sigma-Aldrich (US).

Statistical analysis

Data analysis was performed on three independent experiments. Data are expressed as mean ± SD. Statistical differences in results between groups were evaluated by the Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Results of our study show that administration of BLM into a healthy rat severely inhibits PARP 1 activity in thymocyte nuclei 24 h after drug injection. The activity of the enzyme was restored to a control level in 48 h. We detected that injection of OLA into rats had no appreciable effect on PARP 1 activity in thymocyte nuclei [Figure 1a].

To eliminate the influence of uncontrolled physiologic and pharmacodynamic variables within an organism we examine the effect of treatment with BLM and OLA in a model system containing isolated thymocyte nuclei. The capability of BLM to suppress PARP 1 activity was observed in thymocyte nuclei that were isolated from control group rat thymus and that incubated in the presence of BLM for 2 h [Figure 1b] (*in vitro* settings). When thymocyte nuclei isolated from control group rats were incubated in the presence of OLA, we observed significant inhibition of PARP 1 activity (nearly by 50%) in 2 h, in contrast to the case when OLA was administered to rats *in vivo*.

The duration of isolated thymocyte nuclei's exposure to OLA or BLM was limited by the time when spontaneous DNA fragmentation started. Our preliminary data show that DNA cleavage started 4 h after the beginning of thymocyte nuclei incubation in isolation media (0.25 mM sucrose buffered with 20 mM Tris pH 7.4, containing spermine 15 mM and spermidine 0.5 mM) [Figure 2]. Interestingly, DNA cleavage was not random and resembled internucleosomal DNA fragmentation (apoptotic DNA laddering).

Earlier, it was documented that PARP-1 trapping caused by ATP-induced inhibition of auto-PARylation diminished accessibility to MNase.[15] Coming from the knowledge that the pattern and intensity of DNA olygonucleosomal fragmentation reflect structural changes in chromatin.[16] In the present study, we attempt to determine whether treatment of rats with BLM or OLA can alter olygo nucleosomal cleavage of chromatin and melting profiles of DNA isolated from thymocyte nuclei.

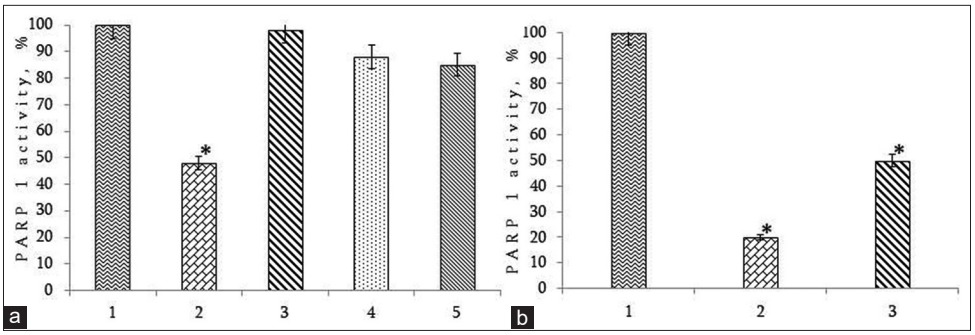

Figure 1: Effect of BLM and OLA on PARP 1 activity in thymocyte nuclei: (a) after administration of BLM (15 mg/1 kg) and OLA (15 mg/1 kg) to rat: 1- control, 2–24 h after BLM administrationto healthy rat, 3–48 h after BLM (1 μM) administration to healthy rat, 4–24 h after OLA administration, 5–48 h after OLA administration; (b) thymocyte nuclei were isolated from healthy rat of control group and isolated nuclei were incubated for 2 h in: 1-nuclei isolation media (control), 2-nuclei incubation media contained BLM (1 μM), 3-nuclei incubation media contained OLA (1 μM).* *P*<0.05

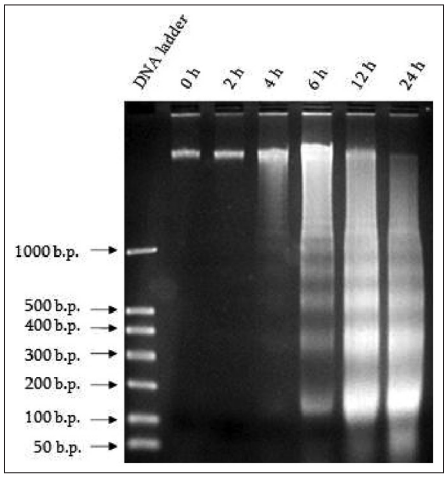

Figure 2: DNA spontaneous internucleosomal fragmentation during incubation of naked thymocyte nuclei in nuclei isolation media

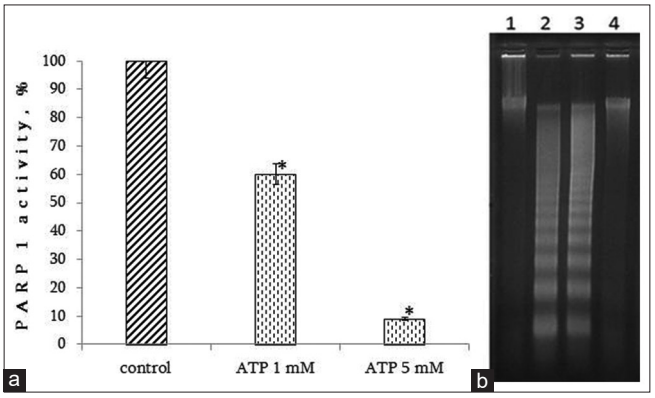

Figure 3: (a) inhibition of PARP1 activity in naked thymocyte nuclei by ATP introduced into nuclei isolation media; (b) - 1- DNA isolated after 60 min incubation in nuclei isolation media. 2- DNA isolated from nuclei in 60 min after addition of $CaCl_2$ (1 mM) and $MgCl_2$ (6 mM) into nuclei incubation media. 3- DNA isolated from nuclei in 15 min after pre-incubation with 1 mM ATP and 60 min incubation with Ca^{2+}/Mg^{2+} ions. 4 - DNA isolated from nuclei in 15 min after pre-incubation with 5 mM ATP and 60 min incubation with Ca^{2+}/Mg^{2+} ions.* *P*<0.05

Herein, we examine whether ATP-induced inhibition of PARP 1 auto-ribosylation in thymocyte nuclei would correlate with chromatin accessibility to Ca^{+2}/Mg^{+2}-dependent endonuclease. Ca^{+2}/Mg^{+2}-dependent apoptotic endonuclease in isolated thymocyte nuclei was artificially activated by introducing $CaCl_2$ (1 mM) and $MgCl_2$ (6 mM) into the nuclei's incubation media.

Data depicted in Figure 3a demonstrate that PARP 1 activity was inhibited in nuclei incubated in the presence of different physiological concentrations of ATP. Inhibition of PARP-1 auto-PARylation by ATP in thymocyte nuclei coincides with suppression of olygonucleosomal cleavage of DNA [Figure 3b] initiated in thymocyte nuclei in 45 min after Ca^{2+} and Mg^{2+} ions addition into nuclei incubation media.

OLA displays high PARP-1 trapping activity.[9] In the present study, we intended to examine whether PARP 1 inhibition in thymocyte nuclei induced by BLM or OLA could exhibit similar changes in chromatin structure as in the case of PARP 1 trapping by virtue of inhibition of auto-PARylation by ATP.

For this purpose, we ran experiments aimed at examining chromatin accessibility to Ca^{+2}/Mg^{+2}-dependent endonuclease after pre-treatment of rats with drugs. The data presented in Figure 4 come to show that high molecular weight (exceeding 1 Kb) fragments of DNA isolated from thymocyte nuclei of rats pre-treated with BLM form a smeared electrophoretic band indicating a random distribution of different length fragments (lane 3), while counterparts from OLA-treated rat thymocytes display a compact band and lack DNA cleavage (lane 4). When thymocyte nuclei from OLA-treated rats were digested with Ca^{+2}/Mg^{+2}-dependent apoptotic endonuclease, the bands corresponding to olygonucleosomal fragments disappeared (lane 6), indicating decreased accessibility of chromatin for endonucleolytic attacks and chromatin condensation. In the case when thymocyte nuclei were isolated after BLM administration to rats, the chromatin accessibility to Ca^{+2}/Mg^{+2}-dependent endonuclease was not significant in regard to chromatin cleavage in control nuclei

(lane 7). Nearly total elimination of olygonucleosomal DNA fragmentation was observed when DNA was isolated from thymocyte nuclei of control group rats that were incubated with OLA in an *in vitro* experimental setting (lane 8). This data come to show that OLA-induced chromatin condensation in thymocyte nuclei occurred both in *in vivo* and *in vitro* experimental settings.

The data come to show that ATP and OLA-induced PARP 1 inhibition in isolated thymocyte nuclei (*in vitro*) correlated with the shift of DNA Tm towards high temperatures [Table 1] and ΔTm widening. Delayed renaturation of DNA and ΔTm widening isolated from thymocyte nuclei incubated with ATP and OLA come to show increased heterogeneity of the DNA. In contrast, BLM-induced PARP1 inhibition correlated with a significant decrease in thymocyte nuclear DNA Tm. In concert with the elevated intensity of DNA internucleosomal cleavage in thymocyte nuclei after Ca^{+2}/Mg^{+2}-dependent endonuclease digestion [Figure 4,] these data suggest BLM-induced loosening and destabilization of DNA.

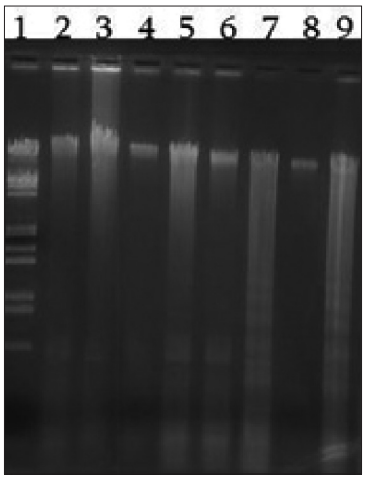

Figure 4: Effect of BLM and OLA on thymocyte nuclei chromatin digestionby Ca^{+2}/Mg^{+2}-dependent endonuclease (1 h,6 mM $MgCl_2$, 1 mM $CaCl_2$).1-λ ladder, 2- control rat, 3- BLM (15 mg/1 kg) treated rat (24 h), 4-OLA treated rat (24 h),5- isolated thymocyte nuclei (control) after Ca^{+2}/Mg^{+2}- endonuclease digestion, 6- thymocytenuclei isolated from OLA (15 mg/1 kg, 24 h) treated rat after Ca^{+2}/Mg^{+2}- endonuclease digestion, 7- thymocytenuclei isolated from BLM (15 mg/1 kg, 24 h) treated rat after Ca^{+2}/Mg^{+2}- endonuclease digestion, 8- control nuclei were pre-incubated 2 h with OLA *in vitro* prior to digestion with Ca^{+2}/Mg^{+2} endonuclease, 9- control nuclei were pre-incubated 2 h with BLM prior to digestion with Ca^{+2}/Mg^{+2} endonuclease

Table 1: Melting temprature of DNA isolated from thymus nuclei incubated with ATP (5 mM), BLM (1 μM) and OLA (1 μM)

	Tm (C°)	ΔTm
Control	51.4	15.9
Incubation media+5 mMATP	61.2*	16.6
Incubation media+1 μM BLM	39.9*	15.6
Incubation media+1 μM OLA	58.3*	16.1

*$P<0.05$

DISCUSSION

BLM, which is widely used in the treatment of germ cell tumors, squamous cell carcinoma, pancreatic cancer, lymphomas, and pleural sclerosis, exhibits severe pulmonary toxicity. BLM therapy cause inflammation of the lung parenchyma and eventually life-threatening pulmonary fibrosis. To maximize cancer cell kill while minimizing BLM toxicities, multi-agent chemotherapy is exploited where treatment with BLM is combined with the PARP1 inhibitor OLA. However, co-treatment with BLM and OLA induces cytotoxicity in cells of healthy bone marrow.[10] Given this context, we suppose that the therapeutic advantage of co-treatment with BLM and OLA should be revalidated considering the challenges that combination chemotherapy presents for healthy cells in cancer patients.

In the present work, we addressed two questions: (1) whether BLM and OLA affect PARP1 activity in healthy rat thymocyte nuclei; and (2) whether OLA and BLM could affect DNA and chromatin structure in healthy thymocytes, based on the fact that OLA increased PARP1 association with DNA in alkylation damage.[9]

Our data demonstrate that administration of BLM to healthy rats leads to PARP1 inhibition in thymocyte nuclei [Figure 1]. PARP 1 inhibition by BLM was also detected in *in vitro* experimental settings where nuclei isolated from control group rats were incubated with BLM in media free of Fe^{++} ions. We suppose that in the absence of Fe^{++}-dependent free radical-initiated DNA cleavage, the DNA minor groove binding properties of BLM[17] become more pronounced and underlie drug-induced PARP1 inhibition in thymocyte nuclei considering, that minor groove binding ligands suppress PARP1 activation.[18]

OLA is recognized as a potent PARP1 inhibitor with an *in vitro* IC_{50} of 5 nM. It is exploited as a monotherapy agent in the treatment of cancers with germline mutations in the DNA repair genes BRCA 1/2 that are required for DNA double-strand break repair and is approved as maintenance therapy in many combination chemotherapy regimens. However, there is no data regarding the impact of OLA on PARP1 activity in healthy cells, though it could underlie a plethora of target-off effects of the drug. Herein, we study whether administration of OLA could modulate PARP1 activity in healthy rat thymocyte nuclei. We observed that, in contrast to BLM, treatment of healthy rats with OLA did not influence PARP 1 activity in thymocyte nuclei. Nevertheless, when isolated thymocyte nuclei were exposed to OLA *in vitro,* we observed more than 40% suppression of PARP1 activity. From this, we speculate that in the context of an organism, the

precise determination of OLA inhibitory potency in healthy cells could be underestimated due to pharmacokinetic, pharmacodynamic, and cell-specific variables determining the drug availability for cell nuclei.

It is considered that binding of PARP to the PARP 1 catalytic domain leads to stabilization of the PARP1-DNA complex (allosteric regulation of PARP-trapping activity).[19] This phenomenon is recognized as PARP1 "trapping" and contributes greatly to the cytotoxicity of PARP clinical inhibitors.[9] OLA exhibits the strongest PARP1 "trapping" efficiency[20] and elicits cytotoxicity even toward healthy bone marrow cells.[10] Considering that ATP-dependent PARP1 trapping on DNA leads to condensation of artificial chromatin constructs[15,21] we were interested in examining whether OLA and ATP could induce modulation of chromatin structure in healthy rat thymocyte nuclei, assuming that the high cytotoxicity of OLA could be partly determined by down-regulation of chromatin-associated vital nuclear functions by virtue of drug-induced chromatin condensation.

The results of our investigation come to show that ATP inhibits PARP1 activity in isolated thymocyte nuclei and chromatin oligonucleosomal cleavage in a dose-dependent manner [Figure 3]. These data are in good agreement with the aforementioned observations documented for artificial chromatin structures and suggest that ATP could be involved in the regulation of PARP1 activity and chromatin condensation in living cells.

Though treatment of healthy rats with OLA did not affect PARP1 activity [Figure 1a], it influenced chromatin packaging in thymocyte nuclei [Figure 4, lane 6]. More pronounced chromatin condensation was detected in isolated thymocyte nuclei of intact rats treated with OLA directly when naked nuclei were incubated in the presence of the drug [Figure 4, lane 8]. Examination of melting profiles of DNA isolated from thymocyte nuclei after their treatment with OLA or ATP revealed that both affect DNA melting temperature and ΔTm nearly in the same manner. Increased ΔTm and a slowdown of DNA renaturation provide evidence that DNA isolated from OLA and ATP-treated thymocyte nuclei of healthy rats was more heterogenic than its counterpart from control thymocyte nuclei. We propose that these data, in concert with results indicating high resistance of chromatin to endonucleolytic cleavage, provide evidence that OLA and ATP can modulate chromatin structure in healthy thymocytes through PARP1 trapping on DNA, which is strong enough to resist DNA isolation procedures.

In contrast to OLA, BLM exhibits strong PARP1 inhibition in thymocyte nuclei both *in vivo* (administration to intact animals) and *in vitro* when the drug is introduced into isolated nuclei incubation media. BLM-induced PARP1 inhibition in thymocyte nuclei is associated with chromatin decondensation and DNA loosening. In 24 h after BLM administration to rats, high molecular band smearing was visible on the DNA electrophoregram, indicating the occurrence of randomly distributed high molecular weight DNA fragments. Thymocyte nuclei digestion by Ca^{+2}/Mg^{+2}endonuclease resulted in intensive olygonucleosomal cleavage, which indicated chromatin decondensation [Figure 4]. When BLM was added to the incubation media of thymocyte nuclei isolated from control group animals, the DNA Tm decreased [Table 1 and Figure 5]. These data come to show that BLM enables destabilization of DNA structure in the nuclei of healthy thymocytes. However, different mechanisms could be responsible for BLM-induced DNA loosening. We suppose that incubation of isolated thymocyte nuclei with BLM in the absence of Fe^{++} ions could induce DNA destabilization via BLM binding in the DNA minor groove,[17] whereas in the case of administration to rats, DNA loosening could result from the development of BLM-induced DNA nicks that relieve torsion stress in the thymocyte nuclei's DNA.

PARP inhibitors are used not only in the treatment of cancer patients but are employed in the therapy of pathologies characterized by inflammation.[22] It appears that exploitation of OLA in mono-agent therapy or in combination with BLM could develop difficulties and raise concerns about life-threatening adverse events arising from the drug's effect on chromatin condensation in healthy cells. On the other hand, BLM-induced PARP1 inhibition raises the question of the rationality of BLM combination with PARP1 inhibitors in cancer treatment.

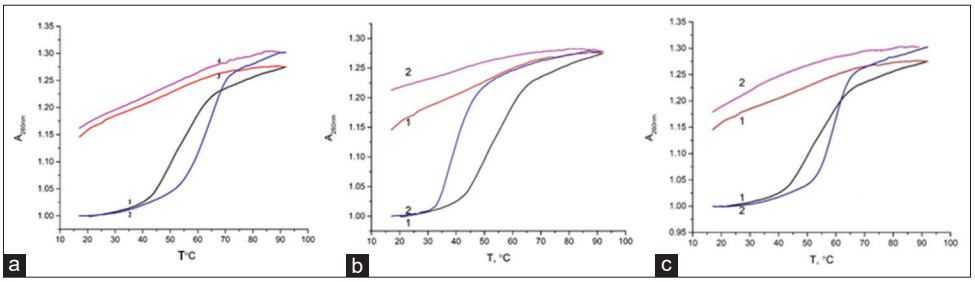

Figure 5: Meltingand renaturationcurves of DNA isolated from control rat thymocyte nuclei; (a) incubatedinisolation media (control), 2- with ATP (5 mM), 3-renaturation curve (control), 4-renaturation curve (5 mM ATP); (b) 1- control, 2-incubated with BLM (1 μM) *in vitro*, 3-renaturation curve (control), 4-renaturation curve (1 μM BLM);c) 1- controlnuclei, 2- incubated with OLA (1 μM), 3-renaturation curve (control), 4-renaturation curve (1 μMOLA)

CONCLUSION

BLM and OLA can affect chromatin structure in healthy thymocytes. OLA-induced chromatin condensation can result in cytotoxicity in healthy cells. Our findings provide evidence for the revalidation of the rationality of OLA application with BLM in combination chemotherapy regimens.

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