ORIGINAL ARTICLE

Effects of Tannic Acid on Cisplatin-Induced Changes in Poly(ADP-Ribose) Polymer Turnover in Rat Liver and Thymocyte Nuclei

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Abstract

Objective: Cisplatin is a powerful antineoplastic drug widely used in the therapy of cancer patients. To attenuate undesirable side effects of the drug, chemotherapeutic regimens are employed based on the use of antioxidant supplementation. The goal of the present study is the examination of the impact of cotreatment with cisplatin and tannic acid (TA) on key members involved in poly(ADP-ribose) polymer (pPARr) turnover in rat liver and thymocyte nuclei, i.e., poly(ADP-ribose)polymerase 1 (PARP 1) activity, poly(ADP-ribose)glycohydrolase (PARG), and NAD^+ content. **Materials and Methods:** Wistar albino rats were randomly distributed in four groups: Control group, treated with cisplatin, tannic acid, and cotreated with tannic acid and cisplatin. The drugs were injected intra-peritoneal. Animals were treated according to regulations of National Centre of Bioethics (Armenia). Cell nuclei were isolated according to standard procedure. PARP 1 activity was evaluated by NAD+ consumption. PARG protein was estimated by sandwich Elisa method. Data are expressed as mean ± s.d. Statistical differences in the results between groups were evaluated by the Student's *t*-test. A probability (*P*) value of < 0.05 was considered significant. **Key Results:** Cisplatin and tannic acid displayed hepatotoxic effects in rat liver in 48 h after treatment. Although treatment of rats either with cisplatin or TA downregulated NAD^+ and PARG content in liver nuclei, the drugs exhibited oppositely directed effects on PARP 1 activity. Cotreatment with cisplatin and TA-stimulated PARP 1 activity in liver nuclei did not affect the basal level of NAD^+ and prevented drastic decrease in PARG protein level. **Conclusions:** Cisplatin-induced inhibition of PARP 1 activity, NAD^+, and PARG content in liver nuclei were eliminated after cotreatment of rats with tannic acid.

Key words: Cisplatin, tannic acid, cotreatment, PARP 1activity, PARG content, NAD^+

INTRODUCTION

Defective mechanisms of DNA repair, which are intrinsic to the vast majority of tumor cells, are widely exploited as a potent pharmacological target for the therapeutic treatment of cancer patients. One of the key events involved in DNA damage repair is coordinated activation of poly(ADP-ribose) polymerase 1 (PARP 1) and poly(ADP-ribose) glicohydrolase (PARG).[1-3]

Poly(ADP-ribos)ylation is one of the earliest and most significant post-translational protein modifications under cellular stress conditions, which modulates chromatin structure. Recently poly(ADP-ribos)ylation of DNA in eukaryotic nucleus was documented, and this observation augmented the knowledge about the role of (ADP-ribos)ylation in the regulation of activation or silencing of chromatin regions.[4]

Poly(ADP-ribos)ylation is mediated by a group of ADP-ribosyl transferases (ADPRT)/poly(ADP-ribose) polymerases (PARPs) discovered over 50 years ago.[5] The enzymes of this group cleave NAD^+ and transfer ADP-ribose moieties to acceptor polypeptide in the form of single unite in the case of mono(ADP-ribosyl)ation. (ADP-ribosyl) polymerases perform further sequential addition of ADP-ribose units to

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first unit attached to protein and form linear or branched ADP-ribose polymers anchored on protein. High negative charge of pADPr chains bound to proteins modify their conformation and alter biological activities.[6] PARP 1 is a profound member of an enzyme family that is localized to the nucleus. It is the second most abundant nuclear protein after histones and is considered to be the greatest NAD^+ consumer in cell. PARP 1 catalyzes poly(ADP-ribos)ylation up to 10% of non-PARP proteins, while approximately 90% of polyADPribose (pADPr) strands become covalently attached to auto modification domain of PARP 1 molecule itself (AM-PARP).[7] To circumvent energetic catastrophe and cytotoxic effects arising from the accumulation of pADPr the turnover of polymer in cells is strictly regulated.[8]

Large body of evidence accumulated hitherto comes to show that pPARr metabolism is affected by different extra- and intra-cellular signals which modulate enzymatic activities of PARP 1 and PARG.

PARG is the main player implicated in pPARr cleavage in the eukaryotic cell, which accomplishes the cleavage of pPARr chains anchored on proteins. The enzyme plays a leading role in the recovery of initial conformation of proteins and pPARr turnover. Coming from the knowledge of well-documented functions of PARG in DNA repair, replication, and chromatin remodeling, PARG inhibition is considered a promising strategy in therapy of cancer and other diseases.[9,10] As early as 1989, Tanuma *et al.* described an inhibitory effect of tannin on purified PARG from human placenta.[11] Tannic acid is naturally occurring water-soluble polyphenol widespread in plant kingdom, and its curative properties are exploited for centuries. TA demonstrated anticancer properties by inducing apoptosis and controlling cancer cell proliferation.[12] Mechanisms underlying antineoplastic activity of TA remain unclear in contrast to advanced knowledge of events underlying antitumor activity of DNA alkylating agents.

Cisplatin [*cis*-diammine-1,1-cyclobutanedicarboxylate platinum(II)] (CP) is one of the most commonly used antitumor drugs and is recognized as a highly effective cytotoxic agent, which forms DNA-adducts due to chemical modification of nuclear DNA. Accumulation of cisplatin in cells of healthy tissues and organs in the course of treatment is responsible for side effects emerging as severe nephropathy, secondary leukemia, thymomas, and other pathologies of hematopoietic and immune systems even considerable time after drug treatment.[13,14] Thus, the curative effect of cisplatin is attenuated by its toxicity and growing resistance of the cancer cells to drugs. Low-dose cytotoxic drug therapy was designed based on the coadministration of non-toxic drugs to cancer patients. This approach improved clinical outcomes and circumvent undesirable side effects of high doses of cytotoxic drugs employed in anticancer treatment so far. In this context, TA is considered as potent agent that increases the solitary effect of cisplatin.[15]

The present study was aimed to study the effect of coadministration of tannic acid and cisplatin on the relationship between PARP 1 activity, PARG, and NAD^+ content in rat liver and thymocyte nuclei.

MATERIALS AND METHODS

Wistar albino male rats (6 weeks old) were used throughout experiments. Animals were obtained from the stock of the animal house of faculty of Biology, YSU. Rats were housed in laboratory conditions (20±2°C) with a light/dark cycle, fed with commercial rat feed *ad libitum* and were given free access to water. Animals were standardized by weight (100 g) and divided randomly into four different groups, three animals each. Control group animals were injected intraperitoneal (i.p.) with saline; cisplatin treated rats were injected (i.p.) with 10 mg/kg of cisplatin; TA treated rats were injected with tannic acid (100 mg/kg); in cotreatment group TA (100 mg/kg) was administered immediately after cisplatin (10 mg/kg) treatment. Rats were killed under light ether anesthesia by decapitation. The procedures were approved by the National Centre of Bioethics (Armenia) and performed according to the International Recommendations (CIOMS, 1985) guidelines.

All reagents were purchased from Sigma-Aldrich.

Livers were collected, and nuclei were isolated according to Hewish and Burgoyne.[16] Sucrose solutions utilized throughout the nuclei isolation procedure were buffered with 20 mM Tris containing 15 mM NaCl, 60 mM KCl, 0,15mM spermine, and 0,5mM spermine.

Determination of endogenous NAD^+ in nuclei

Nuclei from rat liver and thymus were pelleted by centrifugation at 9000 g for 15 min from 10 ml of nuclear suspensions normalized to 1 mg/ml DNA. Supernatants were discarded, and ice-cold 0,5 N $HClO_4$ was added to nuclear pellets. Acid-soluble materials were extracted by vortexing, and insoluble acidic pellet containing proteins was discarded by centrifugation for 15 min 9000 g. Supernatants were moved to fresh tubes and equal volume of 1N KOH was added to neutralize the acid. NAD^+ content was determined in neutralized supernatant, which was clarified by centrifugation at 9000 g 15 min. NAD^+ was determined according to Putt and Hergenrother.[17]

PARG protein content in isolated liver and thymus nuclei was estimated according to recommendations of the manufacturer of PARG, ELISA kit (MyBioSource, Inc.Rat PARG, ELISA kit).

Histopathological procedures

Livers of rats after decapitation were removed and fixed in 10% formalin in saline. Liver was sliced transversely

and paraffin-embedded for light microscopic examination. Paraffin-embedded sections (5 mkm) were dewaxed with xylenes, stained with hematoxylin and eosin.[18] Histopathological changes in organs were assessed in 30 randomly selected tissue sections from each group studied. Slides were observed under Olympus microscope (Model no. BX51).

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 PARP assay mix adapted by us to quantify NAD^+ consumed by isolated nuclei.[17] 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 nuclear suspension was normalized to 1 mg DNA/ml. PARP reaction was initiated by addition of NAD^+ stock solution to 1000 µl aliquot of nuclear suspension (0.5 mM NAD^+ final concentration). The reaction was carried out for 10 min at 37°C, followed by centrifugation at 13 000g, 4°C for 2 min to discard nuclear pellet. 50 µl aliquote samples of supernatant were transferred to the Falcon UV-Vis transparent 96-well plate. NAD^+ quantitation was performed by sequential addition of 2M KOH, acetophenone (20% in EtOH) and 88% formic acid, in accordance with the original assay. The absorbance of PARP assay mix containing 0,5mM NAD^+ was measured at 378 nm. The amount of NAD^+ was determined using NAD^+ calibration curve, and PARP 1 activity was defined as NAD^+ consumed by nuclei in 10 min per mg of DNA.

Statistical analysis

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

RESULTS

Our data come to show that cisplatin in 48 h after injection displayed significant inhibition of PARP 1 activity in liver nuclei. The enzyme activity decreased nearly two-fold. However, treatment with cisplatin did not reliably affect PARP 1 activity in thymocyte nuclei of rats, and it retained the basal level. According to our data treatment of rats with TA activated PARP1 in rat liver nuclei by 40–50%, whereas enzyme activity in thymocyte nuclei was unaffected [Figure 1].

In the present study, we examined the effect of cotreatment with cisplatin and TA on PARP 1 activity in rat liver and thymocyte nuclei. The data demonstrated that when cisplatin was coadministered with TA in 48 h after treatment the activity of PARP 1 in liver nuclei increased nearly by 50%, while enzyme activity in thymocyte nuclei was unaffected [Figure 2].

Determination of intra-nuclear NAD^+ content revealed that the treatment of rats with cisplatin decreased the level of NAD^+ content in liver nuclei by nearly 40%. Injection of TA to rats diminished NAD^+ content by 25% [Table 1]. Cotreatment of rats with TA and cisplatin had no impact on the basal level of NAD^+ content in liver nuclei of rats. NAD^+ content in thymocyte nuclei was stable and resisted impacts of treatment either with TA, cisplatin, or coadministered TA and cisplatin. $^{**}P < 0.05$.

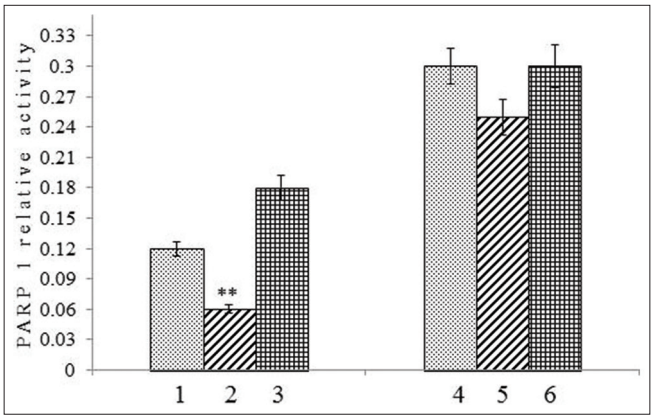

Figure 1: PARP 1 activity in liver and thymocyte nuclei of rats treated with cisplatin in 48 h after injection of the drug. (1) liver nuclei of rats of the control group, (2) liver nuclei in 48 h after treatment of rats with cisplatin, (3) liver nuclei in 48 h after treatment of rats with tannic acid, (4) thymocyte nuclei of rats of control group, (5) thymocyte nuclei of rats in 48 h after treatment with cisplatin, (6) thymocyte nuclei from rats treated with tannic acid. $^{**}P < 0.05$

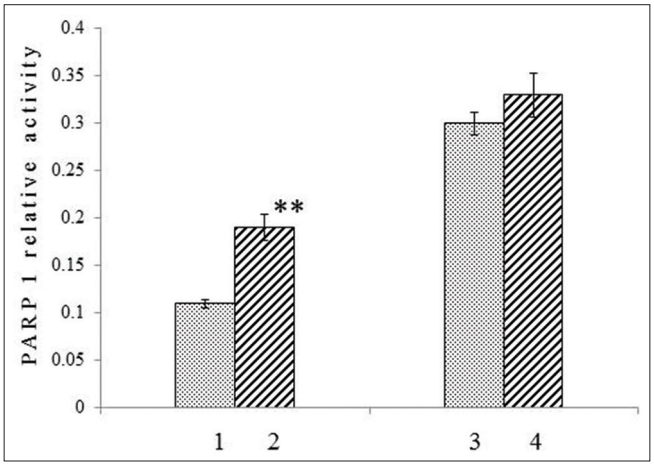

Figure 2: PARP 1 activity in liver and thymocytes nuclei in 48 h after cotreatment of rats with cisplatin and TA. (1) liver nuclei of rats of the control group, (2) liver nuclei in 48 h after cotreatment of rats with cisplatin and TA, (3) thymus nuclei of rats of the control group, (4) thymocyte nuclei of rats in 48 h after cotreatment with cisplatin and TA.

In the present study, we determined the content of PARG protein in liver and thymocyte nuclei of rats treated with cisplatin, TA, and after coadministration of both drugs. Our results indicated that PARG content in liver nuclei of cisplatin-treated rats dropped more than 3,5 fold. Nevertheless, the administration of TA to rats caused a decrease in PARG content nearly to the same extent as treatment with cisplatin, cotreatment of rats with TA, and cisplatin did not affect PARG content. Thymocyte nuclei demonstrated relatively stable level of PARG protein. PARG content in thymocyte nuclei did not change after treatment with either cisplatin, TA, or coadministration of both [Table 2].

The results of histopathological study revealed marked hepatotoxic effects after treatment of rats with cisplatin and TA. Massive fibrosis and steatosis were manifested in the liver of rats treated with either cisplatin or TA. However, after cotreatment of rats with TA and cisplatin, pathologic changes in liver diminished, indicating on the attenuation of hepatotoxic effects of TA and cisplatin [Figure 3].

Table 1: NAD^+ content (μm) in liver and thymocytes nuclei of rats in 48 h after treatment with cisplatin and tannic acid (TA)

Treatment	Liver	Thymocyte
Control	40±3.6	30±2.2
48 h TA	30±3.1	20±2.1**
48 h CP	25±2.8**	26±2.4
48 h (CP+TA)	40±4.0	30±3.1

**$P < 0.05$

Table 2: PARG protein content (ng/mg protein) in liver and thymocytes nuclei of rats treated with cisplatin and tannic acid (TA)

Treatment	Liver	Thymocyte
Control	11.8±2.5	3.1±0.5
48 h TA	3.14±0.4**	2.9±0.5
48 h CP	3.5±0.4**	3.2±0.4
48 h (CP+TA)	10.9±2.0	3.3±0.5

**$P < 0.05$

DISCUSSION

They are mounting evidence come to show that in addition to widely recognized role of NAD^+ in energy metabolism, it is involved in DNA repair, gene expression, and cellular stress response through regulation of NAD^+ consuming nuclear enzymes.[19] As early in 1989, Loetscher suggested that the activity of PARP 1 is related to the availability of NAD^+, and this relationship provides a proper mechanism for tuning nuclear events to the metabolic state of the cell.[20] It was documented that hyperactivation of PARP 1 resulted in NAD^+ and ATP depletion and subsequent energetic collapse, which cause cell death.[21,22] Accumulating data support hypothesis that intranuclear NAD^+ levels affect activities of PARP 1 and sirtuins and serve as a nuclear signal directing cells to survival or death programs.[3] However, the data which directly indicate on the relationship between NAD^+ level and PARP 1 activity in cell nuclei are not yet available. To figure out whether coadministration of TA and cisplatin to rats could affect pPARr turnover in the present study, we examine PARP 1 activity, PARG protein, and NAD^+ content in rat liver and thymocyte nuclei.

The results of our investigations come to show that cisplatin caused dramatic inhibition of PARP 1 activity in liver nuclei in 48 h after administration to rats. We attended to determine whether downregulation of PARP 1 activity in liver nuclei in the group of cisplatin-treated rats correlated with intranuclear NAD^+ content. The results come to show that PARP 1 suppression in liver nuclei of cisplatin-treated rats was paralleled with a decline in NAD^+ concentration, whereas in thymocyte nuclei activity of the enzyme and NAD^+ content were not affected [Table 1]. It was documented earlier that cisplatin

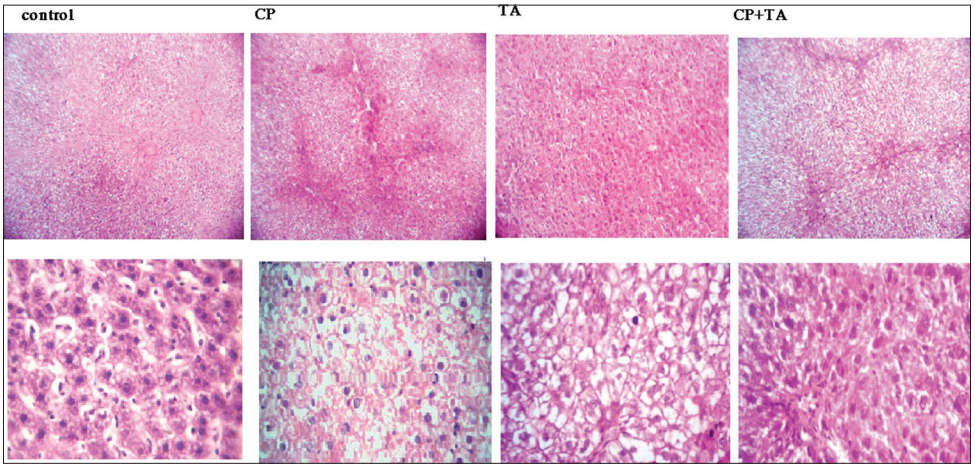

Figure 3: Histopathological changes in liver tissue of rats treated with cisplatin, TA, and after coadministration of cisplatin and tannic acid. Upper panel-magnification ×100; lower panel-magnification ×400.

downregulated hexokinase, phosphofructokinase, and pyruvate kinase activities, which eventually resulted suppression of both anaerobic and aerobic metabolism.[23] Coming from this, we suggested that suppressed metabolic status and downregulation of synthetic pathways involved in NAD^+ synthesis in liver nuclei of rats treated with cisplatin resulted in inhibition of PARP 1 activity. However, we took into account that a drop in NAD^+ level could arise from inhibition of PARG activity and impairment of NAD^+ recycling in a result of "locking" of NAD^+ in PAR chains already generated by PARP 1.[24] To test this possibility, we examined whether cisplatin-induced suppression of metabolism in liver cells could affect PARG synthesis. Coming from this, we determined intra-nuclear concentration of PARG protein in liver and thymocyte nuclei of rats. The results of the study revealed that in 48 h after the treatment of rats with cisplatin, the content of PARG protein in liver nuclei decreased nearly three-fold. We suppose that this data support hypothesis about cisplatin-induced suppression of the metabolic status of liver cells. Concomitantly, changes in PARG content in thymocyte nuclei were unreliable.

Retention of NAD^+ level, PARG protein content, and basal PARP1 activity in thymocyte nuclei of rats treated with cisplatin prompt us that cisplatin possesses organ-specific effect and might differently affect pPARr turnover in organs with different status of metabolic activity.

Histopathological studies of liver collected from rats treated with cisplatin revealed massive fibrosis and steatosis, which supports this speculation.

Our data come to show that when cisplatin was coadministered with PARG inhibitor TA no changes in concentration of NAD^+, PARG, and PARP 1 activity in the liver and thymocyte nuclei occurred in 48 h from the start of treatment. In agreement with these observations, histopathological study shows that cotreatment of rats with cisplatin and TA emerged unexpectedly less hepatotoxicity than mono-treatment either with cisplatin or TA.[25]

The results presented here demonstrated that the treatment of rats with TA led to a decrease in NAD^+ content, PARG concentration, and inhibition of PARP 1 in liver nuclei. On the other hand, our data indicate that cotreatment with TA and cisplatin, downregulated cisplatin, or TA-induced effects on NAD^+ and PARG concentration in nuclei, PARP 1 activity, and hepatotoxicity. To understand the underlying mechanism responsible for antagonistic pharmacodynamical effects of cisplatin and TA in liver cells of rats, we recall the data derived by Tikoo and coworkers, who documented that cisplatin and TA compete for identical binding sites on blood plasma albumins.[26]

CONCLUSION

Our data indicate that cotreatment with TA and cisplatin can lead to decreased bioavailability of drugs, which eventually modulated the content and activity of biochemical components involved in pPARr turnover in metabolically active organs. These results necessitate a further investigation of cisplatin-TA interaction to proceed in the design of harmless chemotherapeutic schemes in cancer treatment.

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