Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• Փորձարարական և տեսական հոդվածներ • Экспериментальные и теоретические статьи •
• Experimental and theoretical articles •

Biolog. Journal of Armenia, 4 (65), 2013

GENDER-DEPENDENT DIFFERENCES IN POLY(ADP-RIBOSE)POLYMERASE-1 ACTIVITY OF RAT LIVER AND THYMOCYTE NUCLEI

I.G. ARTSRUNI, K.S. MATINYAN, A.V. MARGARYAN, A.L. ASATRYAN, E.S. GEVORGYAN

Yerevan State University, Biophysics Department
gana@ysu.am

Gender-dependent differences among therapeutic effects of PARP inhibitors were documented in treatment of various pathologies. The purpose of this study was to examine whether PARP-1 activity displays sexual dimorphism in liver cells and thymocytes of rat. PARP-1 inhibition by benzamide (Bam) and ATP in liver and thymocyte nuclei after in vivo administration of cis-diammine-1,1-cyclobutanedicarboxylate platinum (cis-DDP) to animals was studied. Our data show that female thymocyte and liver nuclei, in contrast to male counterparts, exhibit organ-specific differences in PARP-1 activities. PARP-1 activity of female thymocyte nuclei was unaffected by Bam, while in liver nuclei it was suppressed by 45%. PARP-1 activity of the male rats liver nuclei was completely suppressed by Bam. It was demonstrated that in liver nuclei of male rats treated with cis-DDP PARP-1 activity decreased more than 3 times, whereas in liver nuclei of females it was suppressed only by 45%. In addition, the studies revealed that thymocyte nuclei of both sexes were more resistant to in vivo and ex vivo chemical interventions than liver nuclei. It was shown that in vivo administration of anti-cancer drug cis-DDP is capable to modulate PARP-1 inhibition by Bam in liver nuclei in gender-dependent manner which should be considered for improving therapeutic outcomes in adjuvant cancer chemotherapy by PARP-1 inhibitors.

PARP-1 activity – isolated nuclei – anti-cancer therapies – cisplatin – sexual dimorphism

Պոլի (ԱԿՖ-ռիբոզ)պոլիմերազ-1-ի (ՊԱՌՊ-1) արգելակիչների կիրառությունը խթանում է բազմաթիվ դեղամիջոցների ազդեցությունը: Ներկայացվող աշխատանքը նվիրված է ՊԱՌՊ-1-ի արգելակիչներ՝ ԱԵՖ-ի և բենզամիդի (Բամ), ազդեցության ուսումնասիրությանը առնետների լյարդի բջիջներում և թիմոցիտներում հակաուռուցքային դեղամիջոց ցիսպլատինի (ցիս-ԴԴՊ-ի) ներարկումից հետո: Ցույց է տրված, որ իգական սեռի կենդանիների լյարդի բջիջների և թիմոցիտների ՊԱՌՊ-1-ի ակտիվությունը բավականին տարբեր է: Ֆերմենտի ակտիվությունը արու առնետների լյարդի բջիջ-ներում ավելի զգայուն է Բամ-ի արգելակիչ ազդեցության հանդեպ: Ցիս-ԴԴՊ-ի ներարկումից հետո էգ առնետների լյարդի բջիջների զգայնությունը Բամ-ի արգելակիչ ազդեցության հանդեպ աճում է, իսկ արուների լյարդի բջիջներում ՊԱՌՊ-1-ի ակտիվությունը նվազում է գրեթե երեք անգամ: ՊԱՌՊ-1-ի արգելակիչների ազդեցության փոփոխությունները, որոնք դիտվում են ցիս-ԴԴՊ-ի ներարկումից հետո, չարորակ ուռուցքների բուժման ժամանակ կարող են զգալի ներգործություն ունենալ լրացուցիչ դեղաբուժական միջամտությունների արդյունավետությունը մեծացնելու առումով:

ՊԱՌՊ-1-ի ակտիվություն – մեկուսացված կորիզներ – հակաուռուցքային թերապիա – ցիսպլատին – սեռական դիմորֆիզմ

Применение фармакологических ингибиторов поли(АДФ-рибозо)полимеразы-1 (ПАРП-1) усиливает терапевтический эффект лекарственных препаратов при лечении мно- гих заболеваний. Данная работа посвящена исследованию действия ингибиторов ПАРП-1 АТФ и бензамида (Бам) на активность фермента в тимоцитах и клетках печени крыс до и

после введения интактным животным противоопухолевого препарата цисплатина (цис-ДДП). Показано, что у самок отмечаются орган-специфические различия активности ПАРП-1. Клетки печени крыс самцов более чувствительны к ингибирующему действию Бам. Введение цис-ДДП животным вызывает трехкратное падение активности ПАРП-1 в клетках печени самцов и значительно увеличивает чувствительность клеток печени самок к Бам. Подобные изменения действия ингибиторов ПАРП-1 после введения цис-ДДП могут иметь большое значение для увеличения эффективности палеативного лечения злокачественных новообразований.

Активность ПАРП-1 – изолированные ядра – противоопухолевая терапия – цисплатин – половой диморфизм

PARP-1 is identified as platinum-based drug DNA damage response protein [1, 20]. It was revealed that pharmacological inhibition of enzyme sensitizes cancer cells to chemo- and radiotherapy [2]. Cis-DDP (cisplatin) is one of the most commonly used antitumor platinum-based drug, which forms DNA-adducts due to chemical modification of nuclear DNA. In response to moderate DNA damage caused by cis-DDP, PARP1 is activated improving recruitment of DNA- repair proteins. Massive DNA lesions are responsible for PARP-1 over-activation which triggers apoptotic or necrotic death pathways in cancer cells resulting in overall regression of the tumour [13]. Therefore, PARP-1 is considered as a proper target for pharmacological intervention in cancer treatment, aimed to enhance the cytotoxic effects of antitumor drugs and ionizing radiation [4,11].

However, growing body of evidence comes to show that therapeutic outcomes of treatment with PARP inhibitors in ischemic brain pathologies display marked gender differences, which may arise from distinct cell death mechanisms prevailing in male and female neuronal cells [17]. While majority of investigations is focused on sexual dimorphism in PARP inhibition displayed by neuronal cells, the gender-dependent differences in PARP-1 inhibition in other cells or tissues are less investigated.

It is well known that liver and immune cells are involved in combating toxic invasions of biological and chemical nature. The ability of these cells to process exogenous toxins and chemical insults is influenced by sex-dependent factors [10]. Therefore, in present study it was attempted to determine whether cis-DDP has an impact on PARP-1 activity in rat thymocyte and liver cells and whether enzyme inhibition displays gender-dependent differences in investigated nuclei.

Materials and methods. All reagents were purchased from Sigma.

Albino out-bred rats (6 weeks old, 100-150g weight) were used throughout experiments. Animals were injected with 10 mg/kg cis-DDP intra-peritoneal and decapitated under light ether anesthesia in 48 h after drug administration. Nuclei from liver were isolated according to [5]. Thymocyte nuclei were isolated with slight modifications. Briefly, thymocyte nuclei were not processed through high density sucrose gradient and this step was substituted by several washes in homogenization media. All sucrose solutions utilized throughout liver and thymocyte nuclei isolation procedures were buffered with 25 mM Tris containing 150 mM NaCl, 60 mM KCl, 0,15mM spermine, and 0,5mM spermidine at pH 7, 5.

The enzymatic assay for PARP-1 activity relies on chemical quantification of NAD^+ suggested by Putt and Hergenrother [14].

PARP-1 protein was identified by Western-blot analysis. The nuclear fractions were denatured for 5 min at 95^0C and electrophoresed through 7.5% SDS polyacrylamide gel. The proteins were transferred onto Hybond™ C-Extra nitrocellulose membrane (Amersham).The membrane was blocked in 3% bovine serum albumin (BSA) overnight at 4^0C and was probed with monoclonal anti-PARP-1 antibody (Sigma, clone# C-2-10) diluted 1:1000 in standard incubation buffer (150 mM NaCl, Tris 10mM, pH 7,4, Tween-20 0,05 %) for 2 h at room temperature. Alkaline-phosphatase- linked anti-rabbit IgG (Sigma) (1:30000 dilution, 1 h at room temperature) was used and PARP-1 was detected by NBT/BCIP solution.

Results and Discussion. Direct quantification of NAD^+ consumed by nuclei shows that PARP-1 activity displays organ-specificity in female rats, whereas in male animals no differences in enzyme activity were determined in thymocyte and liver nuclei. Results depicted in fig. 1 show that PARP-1 activity in thymocytes nuclei of female rats is higher than in liver nuclei by approximately 54%.

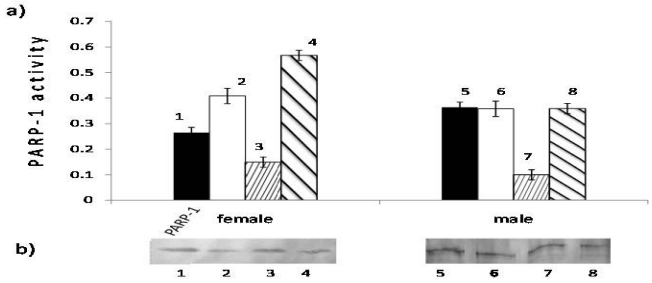

Fig. 1. a) Baseline activities of PARP-1 in: female 1-control liver nuclei, 2-control thymocyte nuclei, 3-liver nuclei after cis-DDP administration, 4-thymocyte nuclei after cis-DDP administration; male 5-control liver nuclei, 6-control thymocyte nuclei, 7-liver nuclei after cis-DDP administration, 8-thymocyte nuclei after cis-DDP administration; b) PARP-1 protein immunodetection in liver and thymocyte nuclei. The numbers in fig.1 correspond to numbers in fig.1a.

Administration of cis-DDP to animals did not affect PARP-1 activity in male thymocytes in 48 h, whereas in liver nuclei enzyme activity decreased dramatically (nearly 3 fold). As it was revealed by immunoblot analyses, the inactivation was not accompanied by PARP-1 molecule cleavage (fig.1b).

In contrast to male liver nuclei, where drastic inactivation of PARP-1 was estimated, administration of cis-DDP to female rats had less dramatic impact on enzyme activity of liver nuclei by decreasing it less than by 44%.

To eliminate the influence of blood-circulating growth hormone (GH) [9] and sex hormones [10] the PARP-1 inhibition in naked thymocyte and liver nuclei ex vivo was investigated. For this purpose the nuclei were isolated from thymocytes and liver cells of animals before and after intra-peritoneal administration of cis-DDP to rats. Inhibitors were added to nuclei incubation media.

The data depicted in fig.2 show that low concentrations of Bam didn't affect PARP-1 activity in rat liver and thymocyte nuclei in both sexes. Marked organ and gender-dependent differences emerged when higher concentrations of Bam were tested. Bam nearly completely suppressed PARP-1 activity in mail liver nuclei, while in female liver nuclei it was less effective (45% inhibition).

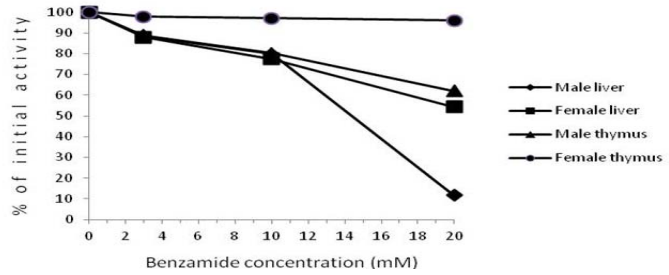

Fig. 2. Inhibition of PARP-1 activity by Bam in liver and thymocyte nuclei of male and female rats.

Thymocite nuclei of both sexes were more resistant regarding to PARP-1inhibition by Bam. Enzyme activity of female thymocyte nuclei was practically unaffected by Bam, while in male thymocyte nuclei it was suppressed by 38%.

In the presence of ATP reduction of enzyme activity in all examined nuclei from animals of both sexes was observed. Nearly complete inhibition of PARP-1 emerged when ATP concentration was raised up to 5mM (fig 3). PARP-1 activity of naked nuclei was inhibited by ATP no matter of sex or organ-specificity.

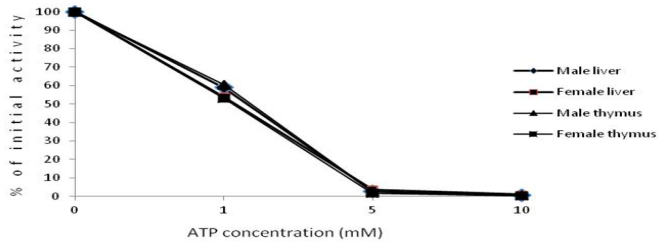

Fig. 3. Inhibition of PARP-1 activity by ATP in female and male rats liver and thymocytes nuclei.

The involvement of PARP-1 in processes determining the choice of cell death pathways as well as cell surveillance necessitates the elucidation of mechanisms underlying regulation of PARP-1 activity in response to cytotoxic stimuli. Intervention with PARP-1 inhibitors enhanced the cytotoxic effects of antitumor drugs and ionizing radiation. [16, 18] and they are considered as a proper adjuvant co-treatment for neuronal decease and cancer therapy. Major evidence demonstrated that in neuronal tissue the therapeutic outcomes of pharmacological inhibition of PARP-1 displayed marked sexual dimorphism. In present study it was examined whether this behavior is unique feature of neurons or is inherent to other cell types as well.

Our data shows that basal activities of PARP-1 in thymocyte and liver nuclei differ significantly in female (54% more in thymocyte nuclei), whereas no-organ specific differences were revealed in mail. Earlier it was shown that testosterone enhanced PARP-1 activity in mice [17]. From the other hand estrogen may be responsible for low PARP-1 activity in female liver nuclei by preventing direct binding of PARP-1 to DNA [12]. It is obvious that PARP-1 activities in different organs in vivo could be maintained by complicated interplay of sex hormones circulating in blood. However, conceiving PARP-1 as one of the most abundant molecules in nuclei (1-2 million copies of PARP-1protein molecule) [15], it is supposed that activated etsrogen receptors can hardly ensure tethering of as many enzyme molecules as should be enough to alter kinetics and overall activity of PARP-1 in nuclei. It is supposed that intrinsic limitations in NAD^+ consumption, hence relatively low basal activity of PARP-1 of female liver nuclei is predicted by impact of estrogen on metabolic activities and energetic demands of hepatocytes.

To improve the knowledge about gender-dependent responses elicited by PARP inhibitors and to eliminate interfering effect of sex hormones on regulation of PARP-1 activity, the impact of PARP-1 inhibitors on enzyme activity in isolated (naked) nuclei ex vivo in the absence of sex hormone income was examined.

Bam is widely recognized PARP-1 inhibitor of first generation which failed to suppress enzyme activity in cultured hepatocytes nuclei [7]. In contrast, the nuclei isolated from mail rats liver display high sensitivity to Bam. Thymocyte nuclei were far less sensitive to inhibition by Bam. Along with relatively low PARP-1 activity displayed by

female liver nuclei they were more resistant to inhibition by Bam than their male counterpart. Thymocyte nuclei of female animals also demonstrated remarkable resistance to PARP-1 inhibition. Though in our study the sex hormones from incubation media were eliminated, it is detected that male liver and thymocyte nuclei were more vulnerable to PARP-1 inhibition by Bam. These data are in good agreement with results published earlier. These authors revealed that inflammatory response in mail mice is modulated by PARP more readily than in female PARP-1 activity and circulating leucocytes were more susceptible to pharmocological inhibition [19]. Based on high susceptibility of liver nuclei of male regarding Bam, it is speculated that female liver cells have higher detoxifying activities, which eventually provide them more effective defense against chemical interventions.

This proposal was supported by results derived from experiments performed with nuclei isolated after administration of cis-DDP to rats of both sexes. After in vivo administration of cis-DDP we detected 3 fold decrease of PARP-1 activity in mail liver nuclei and loss of susceptibility to inhibitory effect of Bam was detected. Immunoblotting data show that inactivation of PARP-1 observed in male liver nuclei after cis-DDP treatment of animals didn't result from PARP-1 cleavage. In contrast, female liver nuclei isolated after drug administration display unexpected high susceptibility to Bam and less obvious decline in PARP-1 basal activity.

To understand underlying mechanisms of phenomenon, first dual nature of PARP-1 activity was addressed. One of the compounds of displayed enzyme activity is strictly dependent on ability of PARP-1 molecule to bind DNA. The second compound relies on properties of catalytically active site in enzyme molecule which is responsible for binding, cleavage of NAD+ substrate and transfer of ADP-ribose residues to other proteins or to itself (autoribosilation) [3]. It is logical to expect that any substance that can compete with NAD+ for binding with enzyme or enzyme-substrate complex will display a role of inhibitor. From the other hand considering DNA as PARP-1 coenzyme, it should be accepted that PARP-1 activity can be modulated by complicated interplay of DNA binding properties of the enzyme molecules, presence of modulators (inhibitors/activators) and amount of available substrate [6].

In addition the problem is even more knotted because it should be taken into account the mechanisms and biochemical events responsible for resistance of hepatocytes to toxic effect of cis-DDP which involve reduced cellular uptake and deactivation by proteins, thiol-containing species as it moves to target DNA in nuclei [11].

Previously it was shown that autoribosylating activity of PARP-1 can be allosterically inhibited by ATP [9]. Investigations reveal that there was large discrepancy between cooperative kinetics of PARP-1 in solution and its cell structure-associated forms [8]. From this viewpoint it was interesting to examine whether physiological range of ATP concentrations can inhibit PARP-1 activity in isolated nuclei which resembles in vivo situation much more closely than any artificial model. It was detected that 5mM ATP nearly completely inhibited PARP-1 activity in all investigated nuclei. This was not surprising due to the that auto(ADP-ribosyl)ation is responsible for more than 90% of total PARP-1 activity.

In vivo administration of cis-DDP to animals of both sexes didn’t modify overall effect of ATP on PARP1 activity or endonuclease resistance.

Introduction of novel therapeutic regimens in clinical trials needs deeper insights in regulation of PARP1 activity regarding to sexual dimorphism. Gender-dependent differences may serve a hitherto unknown factor which can be entangled in regulation of PARP1 activity and fine tuning of cells’ “live or die” decisions and no matter of the underlying mechanisms of sex dimorphism emerged in PARP-1 activities of thymocyte and liver nuclei they should be taken into account in analysis of possible outcomes of pharmacological intervention with PARP-1 inhibitors in different sex.

REFERENCES

1. *Cepeda V., Fuertes M.A., Castilla J., Alonso1 C., Quevedo C., Pérez J. M.* Anti-Cancer Agents in Medicinal Chemistry, *7*, 3-18, 2007.
2. *Chen Y.,Qiao SH., Tukermann J.,Okret S., Jondal M.* Thymus-derived glucocorticoids mediate androgen effects on thymocyte homeostasis. The FASEB Journal, *24*, pp. 5043-5051, 2010.
3. *D'Amours D, Desnoyers S, D'Silva I, Poirier G.* Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem. J., *342*, 249-268, 1999.
4. *Du L., Bayir H., Lai Y., Zhang X., Kochanek P. M., Watkins S. C., Graham S.H., Clark R.S.B.* Innate Gender-based Proclivity in Response to Cytotoxicity and Programmed Cell Death Pathway. J. Biol. Chem. *279*, 37, 10, pp. 38563-38570, 2004.
5. *Hewish D.R.,Burgoyne L.A.* Chromatin sub-structure. The digestion of chromatin DNA at regular spaced sites by a nuclear deoxyribonuclease. Biochem. Biophys. Res.Com., *52*, 2, pp. 475-481, 1973.
6. *Kim M. Y., Mauro S., Gevry N., Lis J. T., Kraus W. L.* NAD-Dependent Modulation of Chromatin Structure and Transcription by Nucleosome Binding Properties of PARP-1 Cell, *119*, pp. 803-814, 2004.
7. *Kirsten E., Jackowski G., McLick J., Hakam A., Decker K, Kun E.* Cellular regulation of poly-(ADP)ribosylation of proteins.I. Comparison of hepatocytes, cultured cells and liver nuclei and influence of varying concentrations of NAD. Exp.Cell Res., *161*, pp. 41-52, 1985.
8. *Kun E., Kirsten E., Hakam A., Bauer P.I., Mendeleyev J.* Identification of poly(ADP-ribose) polymerase-1 as the OXPHOS-generated ATP sensor of nuclei of animal cells . Biochem. Biophys. Res.Com., *366*, pp. 568-573, 2008.
9. *Kun E., Kirsten E., Mendeleyev J. Ordahl Ch. P.,* Regulation of the Enzymatic Catalysis of Poly-(ADP-ribose) Polymerase by dsDNA, Polyamines, Mg^{2+}, Ca^{2+}, Histones H1 and H3, and ATP. Biochemistry, *43*, pp. 210-216, 2004.
10. *Kwekel J.C., Desai V.G., Moland C.L., Branham W.S., Fuscoe J.C.* Age and sex dependent changes in liver gene expression during life cycle of the rat. BMC Genomics, *11*, 675, 2010.
11. *M. Leung, D. Rosen, F. Scott, A. Cesano, D. R. Budman* Poly(ADP-Ribose) Polymerase-1 Inhibition: Preclinical and Clinical Development of Synthetic Lethality Mol. Med., *17*, 7-8, pp. 854-862, 2011.
12. *Mabley J. G., Horvath E. M., Murthy K. G. K., Zsengeller Z.,Vaslin A., Benko R., Kollai M., Szabo C.* Gender Differences in the Endotoxin-Induced Inflammatory and Vascular Responses: Potential Role of Poly(ADP-ribose)Polymerase Activation. Journal Pharmocology& Exp.Therapeutics, *315*, 2, pp.812-820, 2010.
13. *Megnin-Chanet F., Bolle M. A., Hall J.* Targeting poly(ADP-ribose) polymerase activity for cancer therapy. Cell. Mol. Life Sci., *67*, pp.3649-3662, 2010.
14. *Putt K.S., Hergenrother P.J.* An Enzymatic Assay for Poly(ADP-ribose) polymerase-1 (PARP-1) via the Chemical Quantitation of NAD: Application to the High-Throughput Screening of Small Molecules as Potential Inhibitors Analytical Biochemistry, *326*, pp. 78-86, 2004.
15. *Rouleau M.,A. Patel,M. J. Hendzel,S. H. Kaufmann, Guy G. Poirier PARP inhibition: PARP1 and beyond, Nat Rev Cancer. April 10, 4, 293-301, 2010.*
16. *Szabo C., Pacher P., Swanson R.A.* Novel modulators of poly(ADP-ribose) polymerase, Trends Pharmacol Sci. *27,* 12, 626-630, 2006.
17. *Vagnerova K., Liu K., Ardeshiri A., Cheng J., Murphy S.J., Hurn P.D., Herson P.S.* PARP-1 initiated neuronal cell death pathway–do androgens matter? Neuroscience. *166*, 2. pp. 476-481, 2010.
18. *Virag L., Szabo C.* The Therapeutic Potential of Poly(ADP-Ribose)Polymerase Inhibitors Pharm. Reviews, *54*, 3, pp. 375-418, 2002.
19. *Waxman D.J.,Holloway M.G.* Sex Differences in the Expression of Hepatic Drug Metabolizing Enzymes, Mol.Pharmacol., *76*, 2, pp. 215-228, 2006.
20. *Zhu G., Chang P., and Lippard J.S.* Recognition of Platinum-DNA Damage by Poly(ADP-Ribose)Polymerase-1. Biochemistry. *27*,49. pp. 6177-6183, 2010.

Received on 08.10.2013