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, 2 (67), 2015

AGE-DEPENDENT DIFFERENCE IN THYMOCYTE POLY(ADP-RIBOSE) POLYMERASE 1 INHIBITION AFTER THE IN VIVO TREATMENT OF RATS WITH CISPLATIN

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

Yerevan State University, Department of Biophysics
gana@ysu.am

Immunosupression is the most common toxic side-effect elicited by treatment with cisplatin. To improve cytotoxic effect of cisplatin, nowadays poly(ADP-ribose)polymerase 1 (PARP 1) inhibitors are employed in cancer treatment. Driven with the knowledge that age-related thymic involution is a part of immune system degeneration, the enzyme inhibition in thymocyte nuclei of different age rats treated with cisplatin is investigated in the present study. Presented data show that treatment of intact rats with cisplatin had no appreciable effect on pubertal age (4 week old) male thymocyte, whilst elicited elevation of PARP 1 activity by 40% in female counterparts. Administration of cisplatin to young adult females (10 week old) enhanced PARP-1 activity nearly by 60%. It was revealed that administration of cisplatin to rats displayed age-dependent modulation in PARP 1 inhibition by benzamide and ATP in thymocyte. The data presented herein demonstrate that treatment with cisplatin can modulate efficiency of PARP 1 inhibitors in age-and sex-dependent manner.

Cisplatin treatment – PARP 1 inhibition – thymocyte – age-dependent difference

Իմունային համակարգի ընկճումը ցիսպլատինի տոքսիկ ազդեցության ամենահաճախ հանդիպող դրսևորումներից է: Ներկայումս չարորակ նորագոյացությունների բուժման ընթացքում ցիսպլատինի ցիտոտոքսիկ ազդեցության արդյունավետությունը մեծացնելու նպատակով կիրառվում են պոլի(ԱԿՖ-ռիբոզ)պոլիմերազ 1-ի (ՊԱՌՊ 1) արգելակիչներ: Ելնելով նրանից, որ ուրցագեղձի հասակային հետաճը իմունային համակարգի հետադիմության մասն է, մենք հետազոտել ենք ցիսպլատինի ազդեցությունը ֆերմենտի ակտիվության արգելակման վրա տարբեր հասակի առնետների թիմոցիտների կորիզներում:

Տվյալ աշխատանքում ներկայացված հետազոտությունների արդյունքները ցույց են տալիս, որ ցիսպլատինը չի ազդում դեռատի (4 շաբաթական) արու առնետների թիմոցիտների ՊԱՌՊ 1 ակտիվության վրա, մինչդեռ ավելի քան 40 % խթանում է ֆերմենտի ակտիվությունը էգ առնետների թիմոցիտներում: Ցիսպլատինի խթանիչ ազդեցությունը ավելի ուժեղ է դրսևորվում երիտասարդ հասուն (10 շաբաթական) էգ առնետների թիմոցիտներում (մոտ 60%-ով): Ներկայացված արդյունքները վկայում են, որ ՊԱՐՊ 1 արգելակիչների արդյունավետությունը ցիսպլատինի ներգործությունից հետո կախված է առնետների հասակից և սեռից:

Ցիսպլատինի ներգործություն – ՊԱՌՊ 1 արգելակում – թիմոցիտներ – հասակային տարբերություններ

Иммуносупрессия является наиболее часто наблюдаемым токсическим побочным действием цисплатина. Для усиления цитотоксического действия самого цисплатина при лечении онкологических заболеваний в настоящее время применяют ингибиторы поли(АДФ-рибозо)полимеразы 1 (ПАРП 1). Исходя из того, что возрастная инволюция тимуса является частью дегенерации иммунной системы, в данной работе мы исследовали действие цисплатина на эффективность ингибиции фермента в ядрах тимоцитов крыс различного возраста. Представленные в настоящей работе результаты свидетельствуют о том, что цисплатин не имеет заметного действия на активность ПАРП 1 ядер тимоцитов самцов подростко-

вого возраста (4 недели), в то время как активность фермента в тимоцитах самок увеличивается на 40%. В тимоцитах молодых взрослых самок (10 недель) цисплатин вызывает большую активацию фермента (около 60%). Показано, что инъекция цисплатина влияет на эффективность ингибиции ПАРП 1 бензамидом и АТФ. Результаты, представленные в настоящей работе, указывают на то, что действие цисплатина на эффективность ингибиции ПАРП 1 в тимоцитах зависит от возраста и пола крыс.

Действие цисплатина – ингибирование ПАРП 1 – тимоциты – возрастные различия

Thymus is responsible for differentiation and production of immunocompetent thymocytes, playing a crucial role in generation of proper immunological defense in vertebrates. However, this organ undergoes decrease in size along with life span and this is termed as age-related thymic involution. Amounting evidence come to show that age-dependent thymic involution manifest sexual dimorphism [5]. To generate effective immune response and improve therapeutic outcomes in treatment of many diseases age and sex-related peculiarities should be considered. It is recognized that regulation of immune mechanisms that are responsible for inflammatory reactions depends on poly(ADP-ribose) polymerase 1 (PARP 1). PARP 1 is abundant chromatin associated enzyme involved in DNA repair, maintenance of genomic stability, transcription control, cell death and proliferation [1]. Binding of PARP 1 at DNA breaks or regions comprising altered DNA conformation [15] activates the enzyme to create linear or branched polymers of ADP-ribose attached to PARP 1 itself and chromatin proteins at the vicinity of enzyme localization, marking the point to the repair machinery [8]. Nowadays, PARP 1 inhibitors are entering clinical trials to improve curative potential of DNA damaging agents in cancer chemotherapy and benefits therapeutic outcomes in ischemic insults treatment [2, 4].

Driven by the fact that thymus undergo age-related involution, which accelerates when rodents proceed from pubertal age to adulthood, the PARP 1 activity and inhibition in thymocytes after treatment of pubertal age (4 week old) and young adult (10 week old) rats with cisplatin were studied herein.

Materials and methods. All reagents were purchased from Sigma.

Albino inbreeded male and female rats (4 week and 10 week old) were used throughout experiments. Cisplatin was injected abdominal (10mg/1000g wt). Animals were killed in 48 h under light ether anesthesia by decapitation. Nuclei were isolated according to Hewish and Burgoyne [6]. All sucrose solutions utilized throughout liver nuclei isolation procedures were buffered with 20 mM Tris containing 15 mM NaCl, 60 mM KCl, 0,15 mM spermine, and 0,5mM spermidine at pH 7,4.

The enzymatic assay for PARP 1 activity relies on chemical quantitation of NAD^+ in PARP assay buffer [14]. The assay was adapted to quantification of NAD^+ consumed by isolated nuclei.

Briefly, nuclei gently resuspend in 900 µl PARP assay buffer (20 mM Tris, 6mM $MgCl_2$, 1 mM $CaCl_2$, at pH 7.4). PARP reaction was initiated by addition of NAD^+ stock solution to nuclear suspension in PARP assay buffer to 0,5 mM NAD^+ final concentration. The reaction proceeded for 10 min (37^0C) and was stopped by removal of nuclei from reaction mixture by centrifugation at 13 000 g for 2 min. The supernatants were transferred to the wells of Nunc plane-bottom 96-well plate. NAD^+ quantification was performed in 50 µl of supernatant probes by sequential addition of 2 M KOH and 20% acetophenone (in EtOH), yielding final concentrations of KOH, acetophenone and formic acid in accordance with original assay. The absorbance of PARP assay buffer containing 0.5 mM NAD^+ was determined at 378 nm alongside the samples derived from nuclear suspensions and was set as standard. The amount of NAD^+ present in samples of nuclear suspensions in PARP assay buffer was determined by subtraction of test sample absorbance from the standard.

Results and Discussion. It is well recognized that age-related thymic involution is responsible for greater susceptibility to infections in aging organisms [12]. On the other hand, immunosupression is the most common toxic side-effect elicited by treatment with cisplatin. Thus, the impact of cisplatin on PARP 1 activity of the cells which constitute the first line in immune defense e.g. thymocytes, derived from the glands of rats of different age and sex was examined in this study. It was reported previously that resistance of cancer cells to cisplatin is associated with PARP1 hyperactivation which predicts therapeutic benefits of pharmacologic interventions with PARP 1 inhibitors [11]. However, little is known about the impact of cisplatin administration on PARP1 activity and kinetics of enzyme inhibition by PARP 1 with pharmacologic inhibitors in context of drug-drug interaction. Taking into account age-dependent modulations in PARP 1 activity in peripheral blood lymphocytes [10], we were interested to examine impact of the in vivo treatment with cisplatin on PARP 1 inhibition in thymocyte nuclei by benzamide and allosteric inhibitor ATP after administration of drug to rats of different age. Benzamide is well recognized NAD^+-competing inhibitor of first generation and ample of its derivatives nowadays are employed in clinical trials [3]. It was shown, that ATP binds to autoribosylation domain of PARP 1, thereby influencing DNA-binding route of enzyme control in vitro and thus, is recognized as PARP 1 allosteric inhibitor [9]. Coming from this, and to discriminate non-specific effects which arise from Bam impact on glucose metabolism, DNA synthesis and cell viability from kinetic inhibition of PARP 1, we examine PARP 1 inhibition in isolated thymocyte nuclei [13].

In vivo treatment with cisplatin had no appreciable effect on pubertal age (4 week) male thymocyte, whilst elicited elevation of PARP 1 activity by 40% in female counterparts. Administration of cisplatin to young adult females caused more significant PARP 1 activation in thymocyte (nearly by 60%) (fig1).

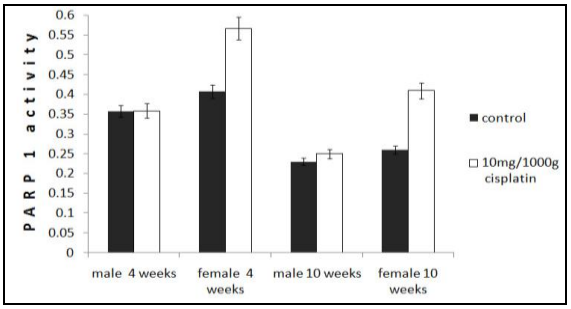

Fig. 1. PARP 1 activity in thymocyte nuclei of rats treated with cisplatin. $p<0.05$

In general, these results come to show that female thymocyte in regard to PARP 1 activity modulation is more susceptible to chemical insult exerted by intervention with cisplatin.

Our data show that PARP 1 inhibition by Bam depends on age and is more effective in thymocyte of 10 week old rats (fig.2, 3).

The results show that there was no difference in PARP 1 inhibition by 1 mM ATP in thymocytes of cisplatin treated 4 week old rats (fig 4, 5). However, thymocytes from 10 week old rats exhibited elevated susceptibility to inhibition by ATP. It was revealed that inhibitory efficiency of 1 mM ATP significantly increased (fig. 6).

The data presented herein demonstrate that treatment with cisplatin can modulate efficiency of the PARP 1 inhibitors in age-dependent manner and in general they are in good agreement with results reported earlier by other authors demonstrating that efficiency of PARP 1 inhibition depends on initial activity of the enzyme [7].

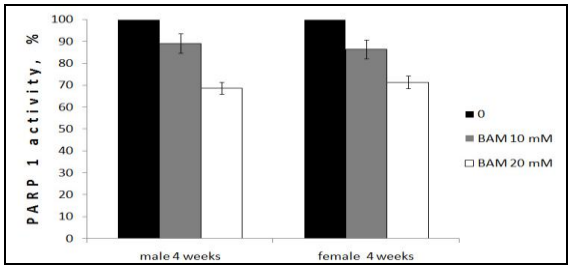

Fig. 2. Inhibition of PARP 1 by Bam in thymocyte nuclei of pubertal age rats injected with cisplatin. Nuclei were isolated in 48 h drug treatment. Bam was added into nuclei incubation media. $p<0.05$.

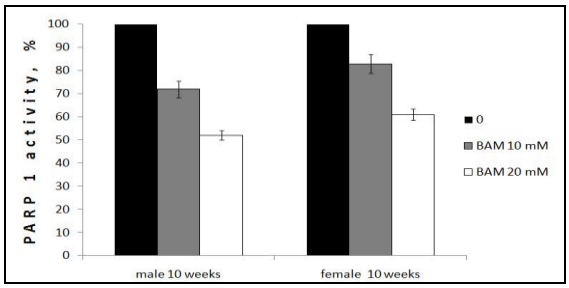

Fig. 3. Inhibition of PARP 1 by Bam in thymocyte nuclei of young adult rats injected with cisplatin. Nuclei were isolated in 48 h drug treatment. Bam was added into nuclei incubation media. $p<0.05$.

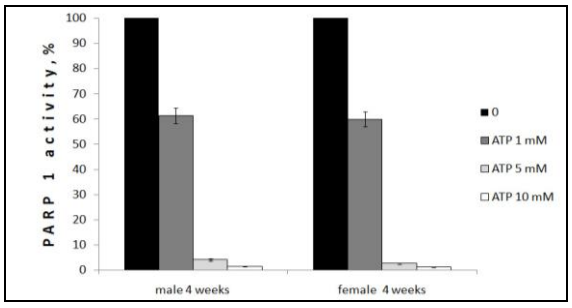

Fig. 4. PARP 1 inhibition by ATP in thymocyte nuclei isolated from pubertal age rats after treatment with cisplatin. $p<0.05$.

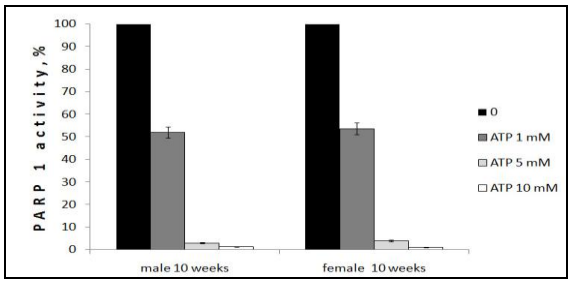

Fig.5. PARP 1 inhibition by ATP in thymocyte nuclei isolated from young adult rats treated with cisplatin. $p<0.05$.

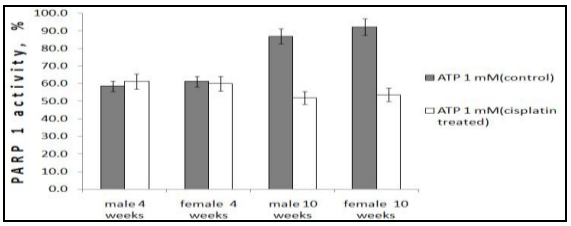

Fig.5. PARP 1 inhibition by 1 mM ATP in thymocyte nuclei isolated from different age rats of control and cisplatin treated groups. $p<0.05$.

Coming from aforementioned, we suggest that age-dependent difference in PARP 1 inhibition after intervention with cisplatin should be considered while designing chemotherapeutic regimen for cancer treatment.

This work was made possible in part by research grant from the Armenian National Science and Education Fund (ANSEF) based in New York, USA

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Received on 26.03.2015