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CHANGES IN PARP-1 ACTIVITY IN RAT LIVER AND THYMOCYTE NUCLEI AFTER IN VIVO ADMINISTRATION OF CISPLATIN

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Cisplatin (cis-DDP)-induced modulations of activity of internuclear nick-sensor enzyme poly(ADP-ribose) polymerase-1 (PARP1) in rat liver cell and thymocyte nuclei were examined. Changes in PARP-1 activity were detected in 48 hours intraperitoneal injection of cis-DDP (10 mg/1000 g body weight). It was shown that in 48 hours of drug treatment the PARP-1 activity in liver cell nuclei was suppressed nearly by 50%, whereas the activity of the enzyme in thymocyte nuclei was enhanced more than two-fold. Our data revealed that cis-DDP-induced modulations of PARP-1 activity in thymocyte and rat liver nuclei were not coupled with apoptotic DNA cleavage.

Cisplatin – nuclei – DNA - PARP-1 activity

Ուսումնասիրվել է ցիսպլատինի (cis-DDP) ազդեցությունը ներ-կորիզային նիկ-սենսորային ֆերմենտ պոլիԱԿՖ-ռիբոզիլ պոլիմերազի (ՊԱՌՊ-1) ակտիվության վրա առնետի լյարդի բջիջների և թիմոցիտների կորիզներում: Cis-DDP (10 մգ/1000 գ) ներարկումից 48 ժ հետո լյարդի բջիջների կորիզներում ՊԱՌՊ-1-ի ակտիվությունը նվազում է, իսկ թիմո-ցիտների կորիզներում աճում ավելի քան երկու անգամ: Ցույց է տրվել, որ ՊԱՌՊ-1-ի ակտիվության փոփոխությունները թիմոցիտների և լյարդի բջիջների կորիզներում չեն զուգակցվում ԴՆԹ-ի ապոպտիկ ճեղքավորումով:

Ցիսպլատին - կորիզ - ԴՆԹ - ՊԱՌՊ-1 ակտիվություն

Исследовано действие терапевтических доз противоопухолевого препарата – цисплатина (cis-DDP) на активность внутриядерного ник-сенсорного фермента поли (АДФ-рибозил) полимеразы 1 (ПАРП-1) клеток печени и тимоцитов крыс.

Показано, что через 48 ч после внутрибрюшинного введения cis-DDP (10 мг/1000 г массы животного) активность фермента в ядрах тимоцитов увеличивается более чем в два раза, в то время как в ядрах клеток печени снижается на 50%. Установлено, что наблюдаемые изменения активности ПАРП-1 не сопряжены с апоптической фрагментацией ДНК.

Цисплатин – ядра – ДНК - активность ПАРП-1

Poly(ADP-ribose) polymerase-1 (PARP-1) is abundant nuclear enzyme and the most elaborated member of PARP enzymes superfamily. The enzyme is implicated in regulation of chromatin structure and is indispensable in a wide range of chromatin-associated processes [11].

PARP-1 possesses activities to cleave NAD^+ and transfer ADP-ribose residues from to γ-carboxy groups of glutamine or, in a lesser extend, to asparagine residues of target proteins including itself (processes are recognized as trans- and auto-poly(ADP-ribos)ylation respectively). These are the common types of PARP activities that account for about 99% of ADP-ribosyl polymers in the living cells. Multiple ADP-ribose units (usually 50-200) can form linear or branched polymers attached to proteins [4].

The PARP-1 activity significantly increases in the presence of single or double-strand DNA breaks and PARP-1 is defined as an effective nick sensor enzyme. PARP-1 protein dimerization is found to be a requisite for high enzymatic activity. However, the DNA-PARP-1-binding stoichiometry and further activation of the enzyme depends upon the character of DNA strand breaks [18]. Investigations comes to show that in the absence of DNA breaks and in physiological conditions the activity of PARP-1 can be modulated by non B forms of DNA e.g. Z conformation, hairpins and cruciforms [15, 21] and various physiologic cellular components [13]. High negative charge of ADP-ribose polymers is responsible for dramatic changes in conformation of acceptor chromatin -associated proteins [9]. Polyribosylation of histone H1 and other chromatin-associated proteins eventually leads to changes in chromatin architecture and activities in the adjacent chromatin regions [12, 14].

The main nuclear target proteins for PARP-1 are p53, NK-kB, histones, topoisomerases, DNA-dependent protein kinase, DNA repairing machinery proteins and endonuclease DNAS1L3 [14, 22]. Involvement of these proteins in regulation of basic nuclear functions determines the crucial role of PARP-1 in cell response to different genotoxic and cytotoxic agents (oxidizing and alkylating agents, UV and ionizing radiation). In the case of moderate DNA damage the PARP-1 activation plays a central role in acute and effective DNA repair which is essential for cell survival. In contrast, massive and unrepairable DNA damage is caused by activation of PARP-1 which lead to rapid depletion of intracellular NAD^+ [20, 23]. In effort to restore NAD content the cells consume ATP. The ability of the cell to retain the ATP content on the level which is sufficient for organized destruction of intracellular structures directs the process of cell death towards apoptotic program, while the failure in ATP production lead to energetic collapse and finally to necrotic death. Thus, PARP-1 determines the choice of death-style of damaged cells in multicellular organisms and plays a role of a molecular switch between different programs of cell demise [6, 8].

In addition to well known role of PARP-1 in basic nuclear functions it was shown, that suppression of the enzymic activity sensitizes cancer cells to ionizing radiation and to a wide range of DNA damaging agents that are widely used in anticancer therapies e.g. cis-DDP, topoisomerase 1 inhibitors and DNA methylating agents [16M. Cis-DDP is one of the most effective and broad-spectrum cytotoxic anticancer drugs. However, arising resistance of cancer cells to the drug undermines its curative potential. To overcome this problem the doses of administrated cis-DDP are gradually increased which leads to manifestation of severe side effects that are originated from cytotoxic features of the drug.

The involvement of PARP-1 in processes of determining the choice of cell death pathways as well as cell surveillance requires the elucidation of mechanisms underlying regulation of PARP-1 activity in response to cytotoxic stimuli. We suppose that this strategy helps to develop new effective approaches in therapies against cancer. Thus, in present study we attempt to examine whether PARP-1 activities in liver cell and thymocyte nuclei of rats are affected after *in vivo* treatment with cisplatin.

Material and Methods. All the chemicals used in this study were obtained from Sigma, USA. Experiments were performed on white, outbred male rats 4-6 weeks old weighting 100-120 grams. Water solution of cis-DDP was injected intraperitoneally (0.5 and 1 mg per 100 g animal weight). Animals were decapitated in 24 and 48 hours drug injection. The nuclei of liver and thymus cells were isolated by Hewish et al [10].

PARP-1 activity was measured by direct quantification of NAD content [19]. The assay was adapted to quantitation of NAD^+ consumed by isolated nuclei.

Briefly, the nuclear pellets were gently resuspended in PARP assay buffer (0,25 M sucrose, 1 mM $CaCl_2$, 6 mM $MgCl_2$, 0,15 mM spermine, 0,5 mM spermidine, 60 mM KCl, 15 mM NaCl, 25 mM Tris, pH 7,4). The density of nuclear suspension was normalized according to DNA content (1mg/ml). 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 8 min (at 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 new tubes and was aliquot (700 µl. NAD^+ quantification was performed in these aliquots by sequential addition of 2M KOH and 20% acetophenone (in EtOH), yielding final concentrations of KOH, acetophenone and formic acid as in original assay [19]. The PARP assay buffer which contained 0,5 mM NAD^+ was set as standard and its absorbance was measured at 378 nm alongside the samples derived from nuclear suspensions. The amount of NAD^+ present in samples of nuclear suspensions in PARP assay buffer was determined by subtraction the absorbance of test sample from the standard.

DNA preparation and electrophoresis was performed by methods described elsewhere [1].

Results and discussion. It is well documented that very often tumorigenesis is coupled with disability of malignant cells to undergo suicide apoptotic program. Recently a new death pathway was established which is induced by poly(ADP-ribose)polymers and is mediated by PARP-1 [17, 24]. Thus, PARP-1-mediated cell death can be enhanced at several points downstream or upstream of PARP-1 activation. Coming from this, the development of drugs that can modulate PARP-1 activity is one of promising approaches in cancer therapy. However, one should take into account that after *in vivo* drug administration the healthy cells undergo drug effects alongside with malignant ones.

This study was devoted to the examination whether cis-DDP has an impact on PARP-1 activity of rat thymocyte and liver cells nuclei. The data shown in Fig. 1 demonstrate that initial PARP-1 activity in thymocyte nuclei is considerably higher than in liver cell nuclei. This data are in good agreement with early report [7], where the highest poly(ADP-ribosyl)ating activity was determined in extracts of rethiculo-endothelial cells, thymus and secondary immune organs.

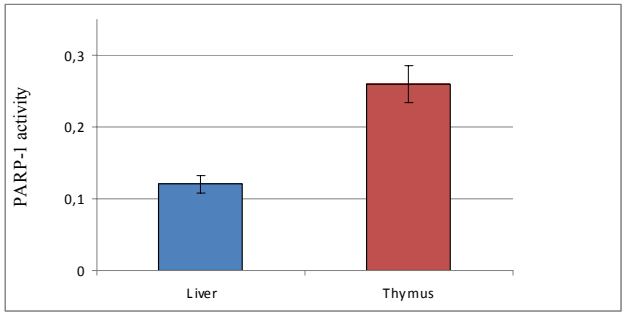

Fig. 1. The baseline activity of PARP-1 in liver cell and thymocyte nuclei.

It should be mentioned that the vast majority of studies devoted to cis-DDP effects were performed on different cell lines and cultures. Common exposure to drug in these experiments does not exceed 24-48 hours. Thus, in present study we examine the effect of cis-DDP on PARP-1 activity of thymocyte and liver cells nuclei after 24 and 48 hours of drug treatment of the healthy rats.

According to our data the low therapeutic doze of cisplatin (5 mg/1000 g) has no appreciable effect on PARP-1 activity in examined nuclei in 24 and 48 hours of drug administration (Fig.2).

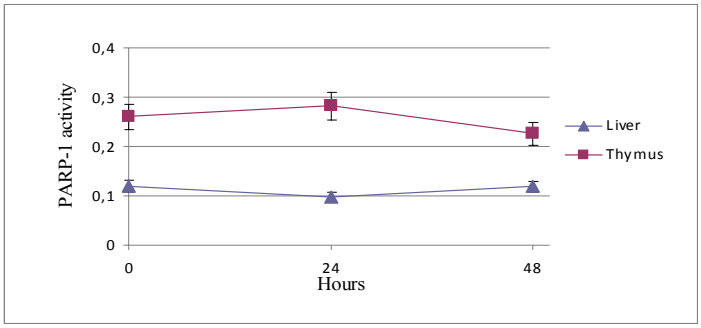

Fig. 2. PARP-1 activity in liver cell and thymocyte nuclei after *in vivo* administration of 5 mg/1000 g of cisplatin

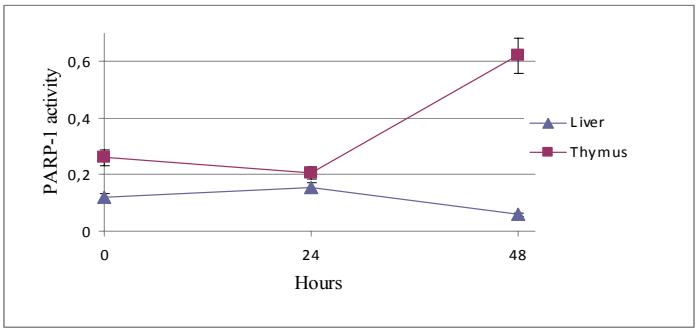

Fig. 3. Changes in PARP-1 activity in liver cell and thymocyte nuclei induced by 10mg/1000g cis-DDP injection

Administration of 10 mg/1000 g cis-DDP doesn't affect the activity of PARP-1 in first 24 hours. However, in further 24 hours i.e. 48 hours of drug treatment with latter doze of drug a drastic (more than two-fold) elevation of PARP-1 activity in thymocyte nuclei was detected.

The changes of PARP-1 activity in liver cell nuclei in 24 hours of high doze cis-DDP treatment were unreliable. After 48 hours of drug administration unexpected decrease (nearly by 50%) of the enzyme activity in nuclei from liver cells was detected (the data shown in Fig.3).

Cis-DDP is recognized as highly effective anticancer drug which triggers multifactorial biochemical responses in cells. Though the mechanisms of cis-DDP cytotoxity are complex, the binding of the drug to genomic DNA and forming of cis-DDP -DNA adducts of different types are recognized as the most prominent steps in the chain of biochemical events that occurred in the cells treated with cis-DDP. Generally, it is considered that the local lesions in DNA structure enhance PARP-1 affinity to DNA, which causes activation of the enzyme. Thus, activation of PARP-1 in thymocyte nuclei, which was detected herein, may be the result of the DNA bending or formation of kinks that were generated by cisplatin-DNA adducts. Nevertheless, our data show that *in vivo* administration of cis-DDP caused inactivation of PARP-1 in liver cell nuclei and these results seems inconsistent to previous supposal. Taking into account that though DNA damage may have significant impact on PARP-1 activity the final outcome is determined by the type of alteration [13] we consider that variations in chromatin architecture of thymocyte and liver cell nuclei can underlay this controversy.

It is documented that the variations in chromatin architecture can maintain formation of different types of cis-DDP-DNA adducts [2] Cepeda et al., 2007], which can in turn, enforce different rotational settings of DNA in nucleosomes [3]. We suppose that changes in DNA positioning enforced by cis-DDP-DNA adducts in liver cell nuclei can differ from those produced in thymocyte nuclei and may foster PARP-1 detachment from chromatin fiber which finally leads to inactivation of the enzyme.

It is well known that apoptosis is one of widely accepted measures of drug cytotoxicity. One of the most characteristic features of biochemical events that underlay execution phase of this cell-suicide program is internucleosomal DNA fragmentation and inactivation of PARP-1 via cleavage by caspase-3, -7. In present study we were interested to examine whether cisDDP-induced suppression of PARP-1 activity was coupled with DNA fragmentation in liver cell nuclei.

Our data show that *in vivo* administration of cisDDP in dozes examined so far didn't cause internucleosomal DNA cleavage in the liver cell (Fig. 4) or thymocyte nuclei (the data are not shown) in 24-48 hours of the drug treatment. Taking into consideration the fact that internucleosomal DNA cleavage is the hallmark of apoptosis we can suppose that *in vivo* treatment of rats with cisDDP doesn't cause apoptosis in examined cells at least in 48 hours of drug administration.

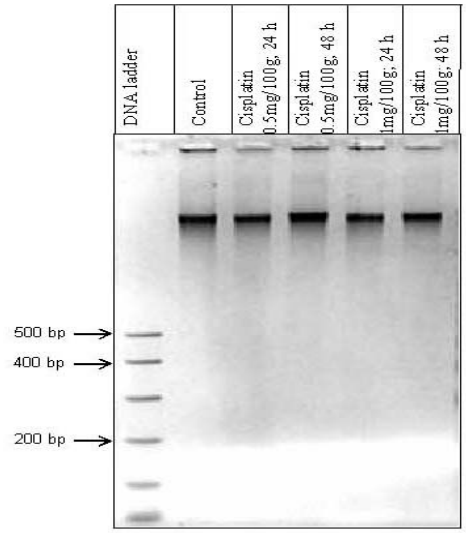

Fig. 4. Rat liver nuclei DNA after *in vivo* treatment of animals with cis-DDP.

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