Research Article

Treatment of Rats with Hydrocortisone and Cisplatin Modulates Poly(ADP-Ribos)yl Polymerase 1 Activity In Liver and Thymocyte Nuclei

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ABSTRACT

Molecular targets of cisplatin [cis-diammine-1, 1-cyclobutanedicarboxylate platinum (II)] are not unique to tumors. The off-target effects of the drug underlie different long-and short-term side-effects and a wide range of health-threatening events. To prevent nausea, vomiting and inflammation cisplatin is often co-administrated with hydrocortisone to cancer patients. Nowadays, when poly(ADP-ribos)yl polymerase 1 (PARP 1) inhibitors are entering into clinical practice is imperative to investigate whether hydrocortisone might be entangled in complex interplay between cisplatin and PARP 1 inhibitors in the course of combination chemotherapy. The results of this study come to show that administration of hydrocortisone to rats improved inhibitory potential of PARP 1 inhibitors (benzamide and ATP) in rat liver nuclei and diminish inhibitory potential of ATP in thymocyte nuclei. Co-administration of cisplatin and hydrocortisone to animals leads to elevation of benzamide inhibitory potential in liver nuclei. Our data come to show that efficiency of PARP 1 inhibitors is modulated by treatment of rats with hydrocortisone in organ-specific manner.

Keywords: Cisplatin, hydrocortisone, co-treatment, rat, PARP 1 inhibition, isolated nuclei.

INTRODUCTION

Cisplatin is recognized as effective cytotoxic DNA-alkylating drug widely employed in cancer therapy. Unfortunately, molecular targets of cisplatin and other drugs used in combination chemotherapy of cancer patients are not unique for tumors and affect healthy cells as well. Many side-effects stem from off-target effects and treatment with cytotoxic drugs is often accompanied by nausea, vomiting and inflammation. For this reason hydrocortisone or other glucocorticoids are usually co- administrated to cancer patients in concert with cisplatin. Cells circumvent the efficiency of DNA-alkylating drugs by activating PARP 1 which is involved in different DNA repair mechanisms. To improve curative potential of DNA-damaging agents several new drugs have been approved for clinical use and PARP 1 inhibitors are indentified as promising drugs which benefit therapeutic outcomes[1-3]. The vast majority of PARP 1 inhibitors are generated as derivatives of benzamide (Bam) the well known PARP 1 competing inhibitor of first generation[4]. The sophisticated mechanism of PARP 1 regulation in nuclei involves ATP. ATP is recognized as PARP 1 allosteric inhibitor, which elicits suppression via binding to auto-parylating domain of the enzyme[5]. Thus, ATP-mimetics which are used in cancer chemotherapy to suppress tumor growth by inhibiting tyrosine kynase-mediated signal transduction pathways are not specific to protein kynases but can elicit off-target effects by competing with ATP for binding with PARP 1 auto-modyfication domain[6]. In present study we were interested to determine whether co-treatment with cisplatin and hydrocortisone can modulate Bam and ATP effects in liver cell and thymocyte nuclei.

MATERIALS AND METHODS

Experimental animal

Animals were treated according to regulations of Committee for Bioethics of Yerevan State University.

Albino inbred healthy male white rats (100-120g, 6 week old) were used throughout experiments. Animals were obtained from the stock of animal house of faculty of Biology, Yerevan State University. Rats were housed in laboratory conditions in polypropelene cages at standard conditions (22±2°C) with a 12 light/dark cycle. Animals were fed with commercial rat feed ad libitum and were given free access to water. The procedures were approved by the National Centre of Bioethics (Yerevan, Armenia) and performed according to the International Recommendations (CIOMS, 1985) guidelines.

Experimental design

In this study the rats were divided into four groups.

Group 1: healthy control rats (C)

Group 2: healthy rats injected with hydrocortisone (5 mg/1000g body weight) (Hyd)

Group 3: healthy rats injected with cisplatin (10mg/1000g body weight) (CP)

Group 4: healthy rats injected with mixture of cisplatin and hydrocortisone (hydrocortisone 5 mg/1000g body weight +cisplatin10mg/1000g body weight) (CP +Hyd).

Drugs were injected intra-peritoneal. First group animals (C) were injected with saline and sacrificed in 48h, second (Hyd) – rats were treated with hydrocortisone and hormone injection was repeated

after 24 hours, animals were sacrificed in 24h after the last injection), third group (CP) - rats treated with cisplatin (single injection, sacrificed in 48h), fourth group (CP+Hyd) were injected with cisplatin and hydrocortisone mixture, hydrocortisone was injected repeatedly after 24 hours (animals were sacrificed in 48h of cisplatin and hydrocortisone mixture administration). Hydrocortisone was injected repeatedly to maintain relatively constant level of hormone in circulating blood for 48 hours.

Nuclei isolation from rat liver and intra-thymic thymocytes

Liver and thymocyte nuclei were isolated according to[7]. Sucrose solutions utilized throughout the nuclei isolation procedure were buffered with 20 mMTris containing 15 mM NaCl, 60 mM KCl, 0,15mM spermine and 0.5mM spermidine, pH 7,4.

PARP 1 assay

The enzymatic assay for PARP 1 activity was performed according to the original method based on estimation of residual NAD^+ concentration in PARP assay mix adapted by us to quantify NAD^+ consumed by isolated nuclei[8]. Briefly, nuclei were gently suspended in PARP 1 assay buffer containing 20mM Tris, 6mM $MgCl_2$, 1 mM $CaCl_2$, pH 7,4. Density of nuclear suspension was normalized to 1mg 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 nuclei consumed NAD^+ for 8 minutes at 37^{0}C and were eliminated from medium by centrifugation at 13 000g, 4^{o}C for 2 minutes. Nuclear pellet was discarded. 50µl aliquot samples of supernatant were transferred to the Falcon UV-Vis transparent 96-well plate. NAD^+ quantification was performed by sequential addition of 2M KOH, acetophenone (20% in EtOH) and 88% formic acid, in accordance with Putt and Hergenrother[8]. Absorbance of PARP assay mix containing 0,5mM NAD^+ was measured at 378 nm. The amount of NAD^+ was determined by using NAD^+ calibration curve and PARP 1 activity was defined as NAD^+ consumed by nuclei in 10 min per mg of DNA.

Isolated nuclei incubation with PARP 1 inhibitors

Isolated rat liver and thymocyte nuclei were resuspended to 1mg DNA/ml in 0,25M sucrose solution buffered with 20mM Tris, 15mM NaCl, 60mM KCl, 0,15mM spermine and 0.5mM spermidine, pH 7,4. Bam or ATP were added into nuclei incubation medium in 15 minutes before addition of divalent ions (final concentrations in reaction mixture were 6mM $MgCl_2$, 1mM $CaCl_2$) and NAD^+ (0,5 mM/ml suspension) to nuclei incubation media. In 8 minutes nuclear pellet was discarded by centrifugation (13000 g, 2min). Residual NAD^+ was determined in 50µl aliquote samples of supernatant.

Statistical Analysis

All results were expressed as M ± S.D from 8 independent experiments. Statistical differences in the results between groups were evaluated by the two-tailed Student's t-test. A probability (p) value of < 0.05 was considered significant.

RESULTS AND DISCUSSION

Modulation of PARP 1 activity in liver nuclei after administration of cisplatin and hydrocortisone to intact animals

The data revealed that treatment of rats with hydrocortisone induced PARP 1 suppression nearly by 15% and 28% in liver and thymocyte nuclei correspondingly. Cisplatin injection to rats led to nearly 2,5-3 fold inhibition of the enzyme in liver nuclei without affecting PARP 1 activity in thymocyte nuclei. After co-administration of cisplatin and hydrocortisone to rats PARP 1 activity in liver nuclei decreased by 38%, whilst in thymocyte nuclei nearly by 21% (Figure 1).

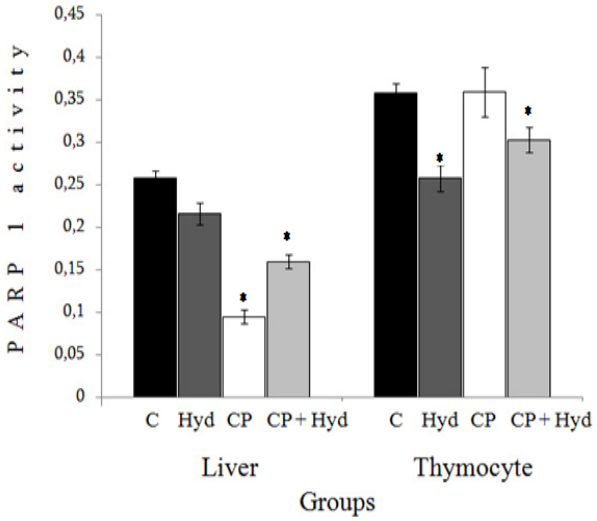

Figure 1: The effect of hydrocortisone (Hyd), cisplatin (CP) and mixture of cisplatin and hydrocortisone (CP+Hyd) injections on PARP 1 activity in rat liver and thymocyte nuclei. *P<0,05

PARP 1 inhibition by Bam and ATP in rat liver and thymocyte nuclei after co-administration of hydrocortisone and cisplatin to animals

To investigate PARP 1 inhibition by Bam and ATP we employed a model system comprising isolated nuclei which closely resembles realistic intracellular situation and circumvents pharmacodynamic effects derived from cisplatin and hydrocortisone superposition in vivo[9-10].

ATP demonstrates powerful inhibitory effect on PARP 1 activity in hydrocortisone-treated rats' liver nuclei. The results of study come to show that ATP more effectively inhibits PARP 1 in thymocyte than in liver nuclei (nearly 40% in thymocyte to 20% in liver nuclei) of control group animals. After hydrocortisone administration to rats PARP 1 was inhibited with 1mM ATP by 55% and 12% in liver nuclei and thymocyte nuclei correspondingly. Treatment of rats with cisplatin has no effect on PARP 1 inhibition by ATP in thymocyte nuclei, whilst improves its inhibitory

potential in liver nuclei (more than 35% inhibition). Liver nuclei of rats which were treated with the mixture of hydrocortisone and cisplatin emerge the same value of PARP 1 inhibition by ATP as the nuclei isolated from rats treated only with cisplatin. PARP 1 inhibition by ATP in thymocyte nuclei is the same as in the case of treatment of rats only with hydrocortisone. The data indicate that 5mM ATP elicits nearly complete PARP 1inhibtion (about 90%) in all examined groups (Figure 2, 3).

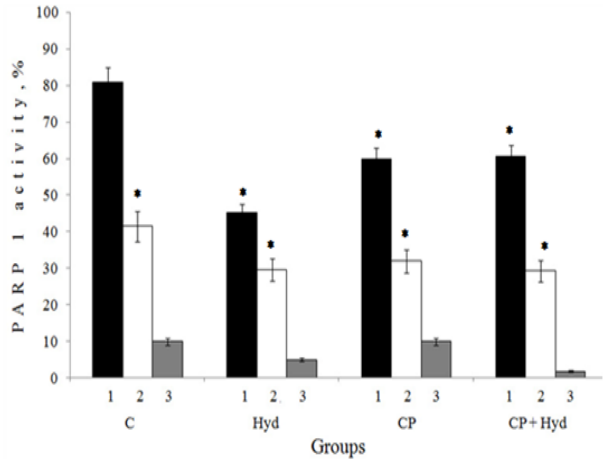

Figure 2: PARP 1 activity in isolated liver nuclei incubated with ATP. Livers were collected from rats of different experimental groups.1- 1mM ATP, 2- 2,5mM ATP, 3- 5mM ATP. PARP 1 activity in nuclei incubated in the media without ATP was set as 100%. *P<0,05

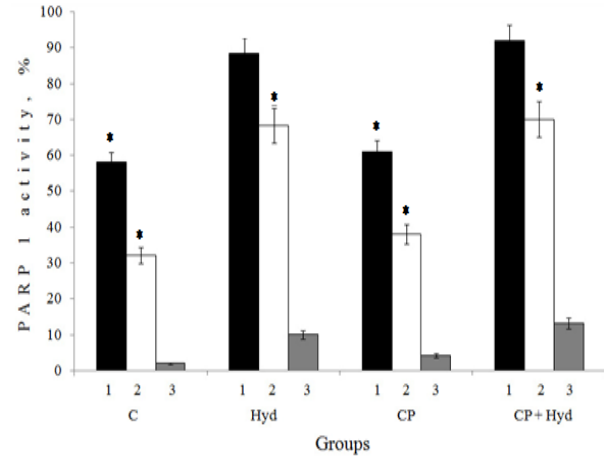

Figure 3: PARP 1 activity in isolated thymocyte nuclei incubated with ATP. Thymuses were collected from rats of different experimental groups. 1- 1mM ATP, 2- 2, 5mM ATP, 3- 5mM ATP. PARP 1 activity in nuclei incubated in the media without ATP was set as 100%. *P<0.05

PARP 1 competing inhibitor Bam elicits nearly the same efficacy in control group animals liver and thymus nuclei (the difference is unreliable). Administration of hydrocortisone to rats has organ-specific effects on PARP 1 inhibition by Bam. Not significant increase in inhibitory potency of Bam in liver nuclei (nearly 45% inhibition to 35% in control group animals) is paralleled with remarkable loss of Bam inhibitory potency in thymocyte nuclei. Cisplatin administration to rats doesn't affect PARP 1 inhibition by Bam neither in liver, nor in thymocyte nuclei. More visible contra-directional changes in Bam efficacy in liver and thymocyte nuclei become apparent after co-administration of cisplatin and hydrocortisone to rats (Figure 4).

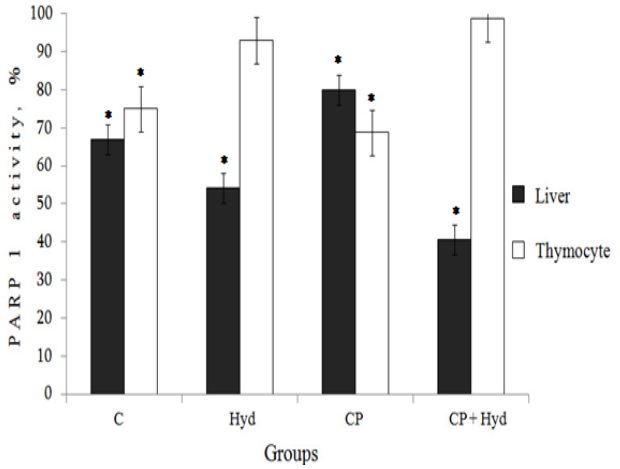

Figure 4: PARP 1 activity in isolated liver and thymocyte nuclei incubated in the presence of 20mM Bam. Livers and thymuses were collected from rats of different experimental groups. *P<0,05

Glucocorticoids (GCs) are steroid hormones that regulate a wide range of physiological functions in organism after they have been released in response to stressful conditions. GCs are the first line drugs that help to sustain homeostasis of organism in situations of undetermined harmful conditions and often they are co-administrated with DNA-alkylating agents in cancer patient chemotherapy to prevent nausea, vomiting and inflammation.

PARP 1 inhibitors are already employed in the clinic situations to augment cancer cell death and the search for more effective ones is in progress. Though many chemotherapeutic regimens encompass treatment with GC, our knowledge about pharmacotherapeutic outcomes of platinum drug, PARP inhibitors and GC superposition is poor. Design of optimal regimens in treatment of cancer patients necessitates advanced understanding of PARP 1 inhibition in context of drug-drug interaction, when combination chemotherapy is employed. Adverse reactions and off-target effects are induced by influence of drugs on normal cells and tissues of organism and are derived from drug superposition in combination chemotherapy. In present study we attempt to determine whether co-administration of cisplatin with hydrocortisone might impact PARP 1 inhibition by Bam (competing) and ATP (allosteric) in liver and thymocyte nuclei of healthy rats.

It is well established that PARP 1 comprises trans- and auto-ribosylating activities[11-12]. Earlier it was reported that PARP 1 is ATP sensor which readily recognizes modulations in intracellular ATP content[13]. It was reported that ATP specifically binds to PARP 1 auto-ribozylating domain thereby, inhibiting auto-ribosylating

activity of the enzyme[5]. Thus, cellular ATP content might determine balance between trans- and auto-parylating activities of PARP 1 and elevation of ATP inhibitory potency indicates on prevailing auto-parylating activity of PARP 1. High inhibitory potency exerted by ATP in thymocyte nuclei indicates on more significant role of enzyme auto-ribosylating activity in thymocyte nuclei in control. Treatment of rats with hydrocortisone leads to increased inhibitory potency of ATP in liver nuclei, in contrast to thymocyte nuclei, where hormone nearly completely eliminates inhibitory effect of ATP. Coming from our hypothesis, and considering that hydrocortisone suppresses mitochondrial energy metabolism and ATP synthesis in liver cells[14], we suppose that hormone administration to rats may gain advantage to PARP 1 auto-parylating activity over trans-parylation in liver nuclei, whereas in thymocyte nuclei the balance between PARP 1 activities is shifted to trans-parylating. In concert, the data of present investigation come to show that treatment of rats with hydrocortisone, cisplatin or with mixture of hydrocortisone and cisplatin changes the balance between PARP 1 auto- and trans-ribosylating activities in organ-specific manner.

Currently the field of PARP inhibitors employment is expanding and as in the case with other chemotherapeutic agents toxicity and acquired resistance are becoming a serious problem. Considering that antitumor activity of PARP inhobitors is associated with platinum drug sensitivity we were interested to examine whether cisplatin and hydrocortisone superposition can affect PARP 1 inhibition by Bam (competing inhibitor)[15]. Bam is prototypic competing PARP 1 inhibitor and the vast majority of modern PARP 1 inhibitors employed in pre-clinical and clinical trials represent a family of Bam analogues that block the binding of NAD^+ to PARP 1catalitic domain, thereby inhibiting both trans- and auto-parylating activity of the enzyme[4,16]. The data of present investigation come to show that treatment of rats with hydrocortisone or co-administration of hydrocortisone and cisplatin decreases Bam inhibitory potency in thymocyte nuclei. In contrast, treatment with hydrocortisone or co-administration of hydrocortisone and cisplatin significantly elevated inhibitory potential of Bam in liver nuclei. Hydrocortisone elicits significant organ-specific differences in PARP 1 inhibition by Bam thus, exhibiting tissue-specific responses.

CONCLUSION

In concert, the data of present study come to show that organ-specific balance between auto-and trans-ribosylating PARP 1 activities can be maintained by external signals (e.g. hydrocortisone or cisplatin administration) and capabilities of PARP 1 inhibitors should be taken into account when enzyme inhibitors are employed in combination chemotherapeutic approaches.

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