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DNA/bovine serum albumin interaction studies and immunomodulatory effects of dinuclear platinum(II) complex with aromatic 1,5-naphthyridine bridging ligand

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Abstract: The DNA and bovine serum albumin binding evaluation of the dinuclear platinum(II) complex, $[\{PtCl(NH_3)_2\}_2(\mu\text{-}1,5\text{-nphe})](ClO_4)_2$ (1,5-nphe is the bridging 1,5-naphthyridine ligand) was investigated by UV–Vis and fluorescence emission spectroscopy. Influence of platinum(II) complex on immune response was also estimated. Peritoneal splenocytes isolated from BALB/c mice were treated with Concanavalin A (ConA) and platinum(II) complex. Concentrations of IFN-γ, IL-1β, IL-17, TNF-α and IL-10 were measured and immunophenotyping was performed. Results showed notable immunomodulatory effects. Pt(II) complex has an inhibitory effect on the release of proinflammatory cytokines in ConA activated splenocytes and $CD3^+$ T cells.

Keywords: immune response; splenocytes; cytokines; platinum(II) complex.

INTRODUCTION

In addition to the widely utilized platinum-based drugs in clinical practice for cancer treatment – cisplatin, carboplatin and oxaliplatin – there is a growing array of compounds still under various stages of development.[1–3] These platinum Pt(II) complexes have been evolving significantly, and research has demonstrated that direct DNA binding, though historically considered the primary mechanism of

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action, is just one of many pathways to achieve antitumor activity.[4,5] This opens up exciting opportunities for new therapeutic strategies.

For instance, Pt-based complexes show potential as photosensitizers in photodynamic therapy, enabling precise targeting of cancer cells when exposed to specific wavelengths of light.[6–8] Additionally, they show promise as immune modulators for chemoimmunotherapy, especially when used in combination with immunotherapeutic agents. Together, these approaches have the potential to enhance anticancer immune responses by synergistically integrating different mechanisms of action.[6]

Such complexes could play a pivotal role in triggering the release and presentation of tumour antigens, which in turn activates immune effectors. This activation may lead to remodelling of the tumour microenvironment (TME), making it less hospitable to cancer growth and increasing overall antitumor efficacy.[6] The multifaceted nature of these complexes' activity makes them particularly appealing for combinatorial treatment approaches.

DNA molecules play a crucial role in regulating cellular functions, making them an excellent drug target, especially in cancer therapy. Reactive ligands can form covalent bonds with DNA or interact non-covalently through electrostatic forces, hydrogen bonding and π–π stacking.[9,1] The primary binding modes include intercalation and minor-groove binding. Minor-groove binding offers higher DNA sequence selectivity and efficiency, targeting AT-rich regions, while intercalation affects DNA conformation and is often directed at GC-rich regions.[9–12] Proteins frequently bind in the major groove, but their biological activity can be influenced by minor-groove binding drugs. Additionally, highly-charged polynuclear platinum(II) complexes represent a novel class of antitumor agents with unique DNA binding mechanisms, such as backbone tracking and groove spanning.[13] The interaction between metal complexes and blood components, such as human serum albumin (HSA), can significantly affect their bioavailability and the activity of biomolecules. Bovine serum albumin (BSA) is frequently used in studies due to its structural similarity to HAS. Investigation of BSA–drug interactions is crucial for understanding of drug activity in organism.

Building on previous findings, where the cytotoxic effects of dinuclear Pt(II) complexes with 1,5-naphthyridine-bridging ligands were demonstrated *in* vitro,[14] further exploration has been conducted to examine their immunomodulatory effects. The results suggest that these complexes not only show promising cytotoxic capacity but could also unlock novel immunotherapeutic avenues, paving the way for innovative cancer treatment paradigms. The interaction of [*cis*-{Pt(L)Cl}$_2$(μ-1,5-nphe)](ClO$_4$)$_2$ complex with calf thymus DNA (CT-DNA) and BSA were examined using UV–Vis absorption and fluorescence spectroscopy techniques.

EXPERIMENTAL

All chemicals and chemical methods used in this study are presented in the Supplementary material to this paper.

Isolation of splenocytes

In order to determinate the effects of Pt-complex on cytokine production by splenocytes derived from healthy BALB/c mouse, isolation of splenocytes was performed according to a previously published protocol.[15] Prepared suspension of freshly isolated splenocytes in complete DMEM medium was placed in 96-well microtiter plate (2×10^5 cells in a well). Based on treatment, there were four groups: control group treated with complete Dulbecco's modified eagle medium (DMEM) medium (complemented with 10 % heat-inactivated fetal bovine serum (FBS), 1 mmol/L penicillin-streptomycin, 1 mmol/L mixed nonessential amino acids (Sigma), and 2 mmol/L L-glutamine) only, platinum(II) complex treated group, Concanavalin A (ConA) treated group (0.5 μg/ml), and ConA and platinum-complex co-treated group. The concentration of the platinum(II) complex used in the experiment was based on its IC_{50} value determined in our previous study.[14] Cells were incubated for 24 h and then their viability was assessed using the trypan blue assay, which confirmed that viability exceeded 90 % in each well, with no differences observed between the groups (data not shown).

Measurement of cytokine concentrations

Cytokine concentrations were measured in supernatants of the cells using Elisa assay kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. Levels of following cytokines were measured: interferon-γ (IFN-γ), interleukins (IL) IL-1β, IL-17, tumor necrosis factor-α (TNF-α) and IL-10. The data are presented as mean ± standard error of the mean (*SEM*).

Flow cytometric analysis of splenocytes

After incubation period, cells were collected and stained with fluorochrome-labeled anti-mouse antibodies specific for CD3 (cluster of differentiation 3) and CD69. For intracellular staining, cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (50ng/ml, Sigma–Aldrich), ionomycin (500 ng/ml, Sigma–Aldrich) and GolgiPlug (BD Pharmingen, NJ) for 4 h and labeled with fluorochrome-conjugated anti-mouse antibodies specific for TNFα, IFN-γ, IL-10, IL-1β and IL-17. FACSCalibur flow cytometer was used (BD Biosciences, San Jose, CA, USA) and the data were analyzed using FlowJo software (Tree Star).

Statistical analysis

The data were analyzed using SPSS (version 23.0). All results were analyzed using Student's t-test or Mann–Whitney U test, where appropriate. Data are presented as mean ± standard error of the mean and statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Dinuclear [*cis*-{$PtCl(NH_3)_2$}$_2$(μ-1,5-nphe)](ClO_4)$_2$ complex, which contains the bridging ligand 1,5-nphe, was synthesized.[14] Fig. 1 depicts the structural formula for the synthesized platinum(II) complex, while the NMR spectra of 1,5-nphe and complex are given in Figs. S2 and S3 (Supplementary material). The interactions of [*cis*-{$PtCl(NH_3)_2$}$_2$(μ-1,5-nphe)]$^{2+}$ complex with DNA were investigated using UV–Vis and fluorescence spectroscopy. Additional studies were focused on the immunomodulatory effect of this complex.

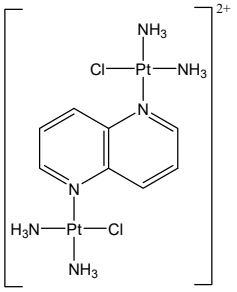

Fig. 1. Structural formula of [*cis*-{PtCl(NH_3)$_2$}$_2$(μ-1,5-nphe)]$^{2+}$ complex.

Interactions of platinum(II) complex with CT-DNA

UV–Vis measurements. The UV–Vis spectra of the [*cis*-{PtCl(NH_3)$_2$}$_2$(μ-1,5--nphe)]$^{2+}$ complex was recorded in the absence and presence of different concentrations of CT-DNA, ([Pt(II)]/[CT-DNA] in 0.0–1.6 range; Fig. 2A). Based on the UV–Vis spectroscopic data, it can be concluded that after the addition of CT--DNA to the solution of platinum(II) complex, a hyperchromic effect occurs, based on which it can be concluded that the complex interacts with CT-DNA. The intrinsic binding constant (K_b) was calculated based on the change in absorbance at 256 nm after the addition of CT-DNA according to the Eq. (S-1) of the Supplementary material and determined from the ratio of the slope of the line and the section on the *y* axis (Fig. 2A, Table I).

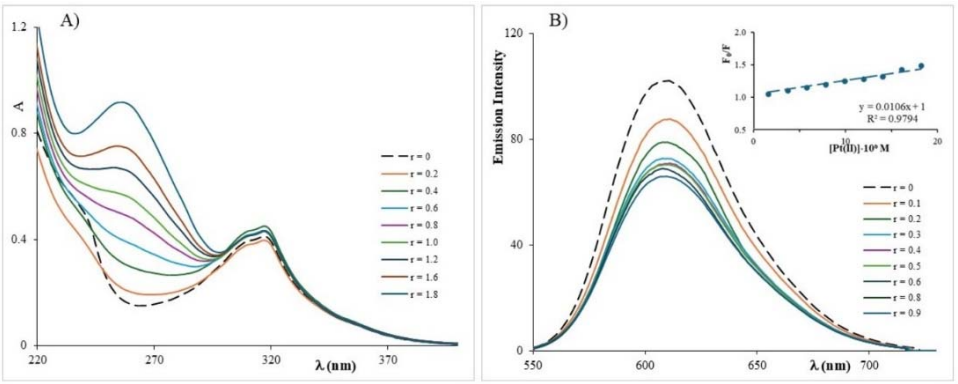

Fig. 2. A) UV–Vis spectra of the Pt(II) complex before and after addition of CT-DNA; B) emission spectra of CT-DNA/EB in the presence of Pt(II) complex (inset: plot of F_0/F *versus* [Pt(II)]; *r* is the mole ratio of Pt(II) and CT-DNA).

Based on the K_b value, it can be concluded that the studied Pt(II) complex, which has a rigid bridging 1,5-nphe ligand with CT-DNA achieves weak interactions. The negative value of the Gibbs energy indicate the spontaneity of the interactions of platinum(II) complex with CT-DNA (Table I). Based on the comparison of the K_b value of the tested complex with K_b of the classic intercalator

ethidium bromide (EB) for DNA, which is 1.23×10^5 M^{-1},[16] it is clear that the K_b value obtained in experiments are lower (≈ 100 times) compared to K_b for EB, based on which it can be concluded that the interactions of the investigated complex is weaker compared to the interactions of EB. The assumption is that the examined complex with CT-DNA achieve interactions that are electrostatic in nature and are a consequence of the interaction of the positively charged complex cation (+2) and the negatively charged phosphate groups in CT-DNA.

TABLE I. Parameters obtained by investigating the reaction of Pt(II) complex with CT-DNA and BSA using UV–Vis and fluorescence measurements; $\%H = 100(A_\infty - A_0)/A_\infty$: hypochromism

Interaction	Method	Parameter	
CT-DNA	UV–Vis	K_b / M^{-1}	1.80×10^3
		ΔG_{298} / kJ mol^{-1}	−19.30
	Fluorescence measurements (EB)	K_{sv} / M^{-1}	1.06×10^4
		K_a / M^{-1}	0.17×10^2
		n	0.3
	Fluorescence measurements (Hoe)	K_{sv} / M^{-1}	0.51×10^4
		K_a / M^{-1}	2.51×10^2
		n	0.5
BSA	UV–Vis	K_b / M^{-1}	7.30×10^3
		$\%H$	71.59
	Fluorescence measurements	K_{sv} / M^{-1}	1.72×10^4
		K_a / M^{-1}	1.70×10^4
		k_q / M^{-1} s^{-1}	1.72×10^{12}
		n	1

Fluorescence spectroscopy. The interactions of Pt(II) complex with CT-DNA were investigated using emission fluorescence spectroscopy. Ethidium bromide (EB) acts as an intercalating agent by inserting itself between adjacent base pairs within the DNA double helix, resulting in strong fluorescence emission at 612 nm when excited at 527 nm, due to the formation of the CT-DNA/EB adduct. In contrast, Hoechst 33258 (Hoe) was used as a marker for minor groove binding, producing a fluorescent adduct that emits intensely at 486 nm upon excitation at 346 nm.[17] Hence, the mode of interaction between the platinum(II) complex and CT-DNA can be assessed by monitoring changes in the fluorescence emission spectra of CT-DNA/EB and CT-DNA/Hoe aducts upon the incremental addition of the platinum(II) complex.

Fig. 2B shows the emission spectra of EB/CT-DNA in the presence of the Pt(II) complex. The addition of the Pt(II) complex solution leads to a decrease in the emission intensity at 612 nm, which indicates that there is a competitive reaction between EB and the examined Pt(II) complex in relation to CT-DNA. After the addition of the Pt(II) complex solution, the emission intensity at 612 nm decreases slightly, which indicates a very weak binding of the examined Pt(II) com-

plex to CT-DNA. The intensity of the interaction of the Pt(II) complex with CT--DNA was determined based on K_{sv} value obtained by using Eq. (S-2). The obtained results are shown graphically as the dependence of F_0/F on [Pt(II)] (Fig. 2B). The Stern–Volmer constant was determined from the slope of the obtained line using the Eq. (S-2). The obtained value for K_{sv}, as well as for the stability constant (K_a), which was determined using Eq (S-3) (Table I), indicate that the tested complex is not good intercalator and that it has weak interaction with CT-DNA.

The DNA binding constant (K_a) of the investigated platinum(II) complex was found to be 0.17×10^2 M^{-1}, which is significantly lower than that reported for a structurally related complex containing a 1,6-naphthyridine (1,6-nphe) ligand (K_a = 5.3×10^4 M^{-1}).[18] This difference suggests that the position of the nitrogen atoms in aromatic naphthyridine ligands provide different interaction with DNA.[17] In addition to the binding constant determined in this study, previously published molecular docking results provided further insight into the interaction between the complex and DNA, indicating that the binding is predominantly electrostatic in nature.[14] This finding is consistent with the moderate binding constant observed experimentally and supports a non-intercalative binding mode.

The fluorescence emission spectra of the CT-DNA/Hoe in the absence and presence of increasing concentrations of the platinum(II) complex are shown in the Fig. S-4 of the Supplementary material. The gradual decrease in fluorescence intensity observed after adding the platinum(II) complex indicates its interaction with CT-DNA/Hoe. The obtained constant (K_a, Table I) values suggest that the studied complex is unable to effectively compete with the minor groove binding agent Hoe ($K_a \approx 1.4\times10^5$ M^{-1}).[19] Based on UV–Vis and fluorometric measurements, it can be concluded that the interactions between platinum(II) and CT-DNA occur from interactions between the positively charged complex ion and the negatively charged phosphate groups in DNA (electrostatic interactions).

Interactions of platinum(II) complex with BSA

UV–Vis spectroscopy. UV–Vis spectroscopy is applied to investigate conformational changes of proteins after their interaction with drugs or potential drugs.[20] In the UV–Vis spectrum of BSA a very strong absorption band in the 220––240 nm region can be observed. The absorption peak of lower intensity at 278 nm originates from the presence of aromatic amino acids (tryptophan, tyrosine and phenylalanine) in the BSA molecule.[21] Therefore, this method is very sensitive to conformational changes of the BSA protein, as well as to changes in the microenvironment of aromatic amino acids. The UV–Vis spectra of BSA in the absence and presence of the Pt(II) complex are given in Fig. S-5A of the Supplementary material. After the addition of Pt(II) complex to the BSA solution, there is an increase in the absorption intensity at 278 nm, on the basis of which it can be concluded that the Pt(II) complex interact with BSA. These changes can be attributed to

electronic transitions of aromatic amino acids. In the examined reactions, a hyperchromic shift of the absorption maximum at 278 nm occured. All this indicates that there are structural changes in BSA due to non-covalent interactions, which may be due to hydrogen bonding or electrostatic interactions.[22] The value of the K_b was obtained on the basis of Eq. (S-4) (Table I) and calculated from the graphical dependence of $(A_\infty–A_0)/(A_x–A_0)$ on 1/[Pt(II)]. Based on the obtained value of the constant, it can be concluded that the Pt(II) complex with BSA achieves strong interaction, which is a consequence of the presence of two condensed aromatic rings in the bridging 1,5- nphe ligand.

Fluorescence spectroscopy. The interactions of the Pt(II) complex with BSA were investigated using emission fluorescence spectroscopy. The solution of BSA gives intense fluorescent emission at $\lambda_{em.max}$= 352 nm, and when excited at 295 nm. After the addition of the Pt(II) complex to the BSA solution, it was observed that with increasing concentration of the complex, there is a decrease in fluorescence intensity (Fig. S-5B) indicating that complex interacts with this biomolecule. Changes in the emission spectra of BSA/Pt(II) complex indicate protein interactions with the complex, which lead to changes in the secondary structure of BSA.[23] Based on the decrease in BSA emission intensity with increasing concentrations of the Pt(II) complex, using the Eqs. (S5) and (S3) of the work, the following were determined: K_{sv}, k_q, K_a, as well as n (Table I). The average fluorescence time of BSA in the absence of the complex is $\tau_0 = 10^{–8}$ s, on the basis of which the fluorescence "quenching" rate constant (k_q, $M^{–1}$ $s^{–1}$) was calculated. The K_{sv} and k_q values indicate that the tested complex can bind to BSA. The fluorescence quenching constant is greater than 10^{10} ($k_q > 10^{12}$ $M^{–1}$ $s^{–1}$) indicating a static emission quenching mechanism.[24] The value of K_a and n were calculated based on Eq. (S3). Based on the K_a value, it can be concluded that Pt(II) complex can bind to BSA. The K_a value for the investigated complex is smaller compared to the K_a for avidin ($K_a = 10^{15}$ $M^{–1}$), which achieves the strongest non-covalent interactions with biomolecules, so the bond of the probe complex with BSA can be easily broken even before reaching the target site in the cell. Complex Pt(II) contains a 1,5-naphthyridine ligand with great steric hindrance resulting in weaker hydrogen bonding with BSA. The number of binding sites for Pt(II) is $n \approx 1$.

Lipid–water partition coefficient (log P*)*. Drug lipophilicity represents a key pharmacokinetic parameter due to its crucial influence on the compound's ability to traverse multiple layers of cells. Lipophilic drugs are typically associated with enhanced biological activity, faster metabolic processing and elimination, as well as stronger binding to plasma proteins.[25] The determined log P value of the investigated complex (log P = –0.86) indicates its hydrophilic nature. This result aligns well with previously reported data for similar platinum(II) complexes.[26] Incorporating aromatic, nitrogen-containing heterocyclic bridging ligands into the coordination environment of platinum(II) notably enhances the complex's hydrophobicity.

Immunomodulatory effects

As the cytotoxic activities of the dinuclear Pt(II) complexes 1,5-naphthyridine-bridging ligand were confirmed *in vitro*,[14] we further examined the immunomodulatory effects of the complex with promising cytotoxic capacity.

First, cytokine production from splenocytes that were incubated for 24 h in medium only, medium with Pt(II) complex, ConA or co-stimulated with Pt(II) complex and ConA were analyzed (Fig. S-6A).

Treatment with Pt(II) complex significantly increased concentrations of IL-1β ($p = 0.001$), IL-17 ($p = 0.004$), TNF-α ($p = 0.008$) and IL-10 ($p = 0.008$) compared to untreated splenocytes (Fig. S-6). ConA is an antigen-independent mitogen and leads to polyclonal proliferation and activation of T cells.[27] ConA significantly increased the concentration of proinflammatory cytokines IFN-γ ($p = 0.001$), IL-1β ($p = 0.001$), IL-17 ($p = 0.001$), TNF-α ($p = 0.008$), and anti-inflammatory IL-10 ($p = 0.008$) in cell supernatants in comparison to untreated cells.

Co-stimulation of splenocytes with Pt(II) complex and ConA significantly reduced concentrations of IL-1β ($p = 0.007$) and TNF-α ($p = 0.008$) compared to ConA. After Pt(II) complex and ConA co-treatment decreasing in concentrations of IFN-γ, IL-17 and IL-10 were also observed, but without statistical significance. Also, we observed ratios of cytokine production- Pt(II) complex/medium and Pt(II) complex + ConA/ConA (Fig. S-6). According to the results, the Pt(II) complex is more likely to exhibit its immunomodulatory effects on non-activated spleen cells, stimulating splenocytes to produce IL-1β, IL-17, TNF-α and IL-10. *Vis-a-vis*, co-treatment with Pt(II) complex and ConA significantly reduced production of IL-1β and TNF-α. IL-1β is cytokine with strong inflammatory and immune-enhancing effects, mainly produced by leukocytes, especially monocytes and macrophages and plays a key role in inflammation, fever, and lymphocyte activation.[28] TNF-α cytokine is engaged in a promotion of inflammatory responses and is responsible for signaling events that lead to induction of apoptosis.[29] Beside inhibitory effect on the release of proinflammatory cytokines after ConA stimulation, it could be also concluded that immunomodulatory effects of Pt(II) complex were more prominent in non-activated cells, opening up the possibility that the investigated complex exerts effects on other splenocytes beside T cells. In order to determinate the relationship between proinflammatory and anti-inflammatory mediators in all groups we analyzed ratio of values of IFN-γ, IL-1β, IL-17, TNF-α with values of IL-10 (Fig. 3). ConA significantly decreased the ratios of IFN-γ/IL-10 ($p = 0.001$), IL-17/IL-10 ($p = 0.001$) and TNF-α/IL-10 ($p = 0.001$) compared to medium (Fig. S-6A, C and D). Also, Pt(II) complex significantly decreased ratio of IFN-γ/IL-10 ($p = 0.001$), IL-1β/IL-10 ($p = 0.002$), IL-17/IL-10 ($p = 0.001$) and TNF-α/IL-10 ($p = 0.001$) compared to medium. Co-treatment didn't significantly affect ratios of pro and anti-inflammatory cytokines compared to ConA. Based on these results Pt(II) complex could upregulate production of anti-inflammatory

cytokine IL-10. IL-10 is an anti-inflammatory cytokine that plays key role in limiting host immune response to pathogens.[30] IL-10 limits inflammation and enhances humoral immune responses. In IL-10-deficient mice, severe intestinal inflammation occurs, which can be alleviated by administering IL-10.[31] Considering that IL-10 plays a key role in preventing inflammatory and autoimmune pathologies,[32] results suggest that Pt(II) complex might have beneficial effects in treating autoimmune diseases.

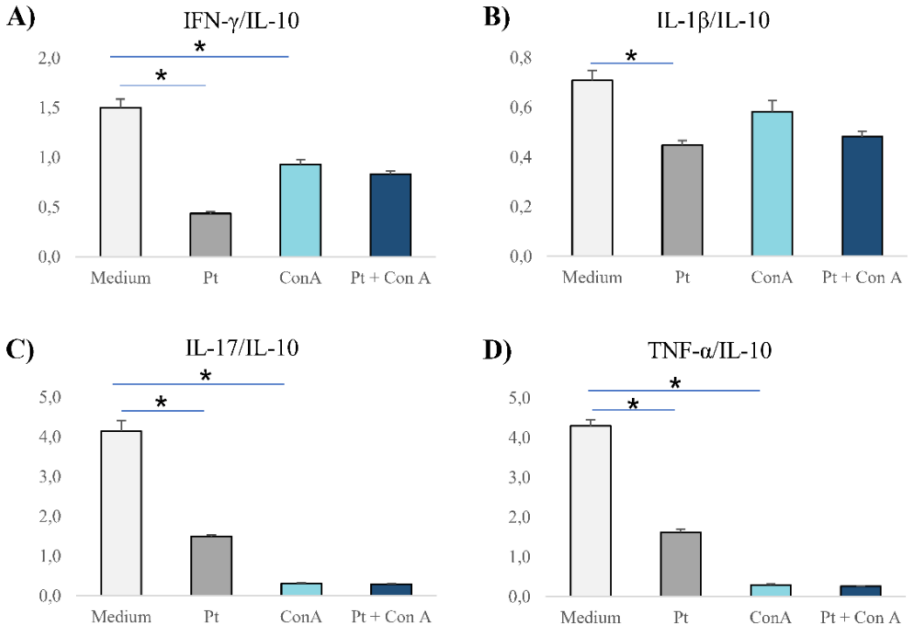

Fig. 3. Ratio of pro- and anti-inflammatory cytokine concentrations produced by splenocytes derived from healthy BALB/c mice after 24-h of incubation with DMEM medium only, Pt(II) complex, ConA, or a combination of Pt(II) complex and ConA. The data are shown as mean ± *SEM*. Statistical significance was determined by student's *t*-test and Mann–Whitney U test, where appropriate (*$p < 0.05$).

With the intention of further understanding the immunomodulatory effects of Pt(II) complex, the functional phenotype of $CD3^+$ splenocytes was analyzed using flow cytometry (Fig. 4A). CD3 complex is the subunit of T-cell receptor (TCR) and it's responsible for signal transduction necessary for activation of the T cells.[33] Activation and presence of $CD3^+$ T lymphocytes is important prognostic factor for various types of cancer so potent immunomodulatory role of Pt(II) complex could contribute to its antitumor effects.[33,34] ConA stimulation significantly increased the percentage of $CD69^+$ ($p = 0.029$), IFN-γ^+ ($p = 0.001$), IL-1β^+ ($p = 0.001$), IL-17$^+$ ($p = 0.001$), TNF-α^+ ($p = 0.029$), IL-10$^+$ ($p = 0.029$) $CD3^+$ cells when compared to medium treated $CD3^+$ cells. Cultivation with Pt(II) complex led to sig-

nificantly elevated percentage of $CD69^+$ ($p = 0.029$), IFN-γ^+ ($p = 0.017$), IL-17^+ ($p = 0.018$), TNF-α^+ ($p = 0.029$) $CD3^+$ cells in comparison with medium treated $CD3^+$ cells. CD69 is transmembrane type II C-lectin receptor and serves as an early indicator of lymphocyte activation. It is important for managing the differentiation of regulatory T cells (Tregs) and secretion of cytokines such as IFN-γ, IL-17 and IL-22.[35] It has been shown that increment of $CD69^+$ T cells and significant increase in cytokines like: IFN-γ and TNF-α could be important for optimal anti--tumor response. Co-stimulation with Pt(II) complex and ConA significantly decreased percentage of $CD69^+$ ($p = 0.029$), IFN-γ^+ ($p = 0.003$), IL-$1\beta^+$ ($p = 0.002$), IL-17^+ ($p = 0.001$) and TNF-α^+ ($p = 0.029$) $CD3^+$ cells compared to ConA treated group. These results suggest that Pt(II) complex may reduce production of proinflammatory cytokines in previously ConA activated $CD3^+$ splenocytes. Also, Pt(II) complex/medium and Pt(II) complex + ConA/ConA ratios of cytokine production by $CD3^+$ splenocytes indicated that Pt(II) complex has pronounced effect in non-activated $CD3^+$ splenocytes.

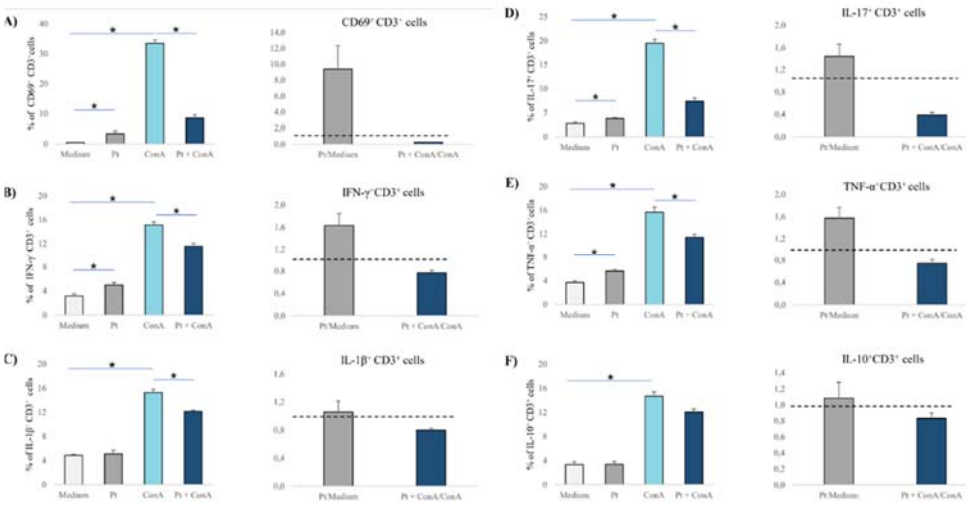

Fig. 4. Effects of Pt(II) complex on cytokine production of CD3+ splenocytes. The graphs show the percentage of CD3+CD69+ (A), CD3+IFN-γ+ (B), CD3+IL-1β+ (C), CD3+IL17+ (D), CD3+TNF-α+ (E) and CD3+IL-10+(F) splenocytes derived from healthy BALB/c mice after 24-h of incubation with medium only, Pt(II) complex, ConA, or a combination of Pt(II) complex and ConA. Cytokine production ratios in splenocytes treated with Pt(II) complex/medium compared and treatment with Pt(II) complex + ConA/ConA were presented (A–F). The data are shown as mean ± *SEM*. Statistical significance was determined by student's t-test and Mann–Whitney U test, where appropriate (*$p < 0.05$).

CONCLUSION

The DNA-binding evaluation of Pt(II) complex was carried out by UV–Vis and fluorescence emission spectroscopy. Electrostatic interactions between the positively charged platinum(II) complex and negatively charged DNA backbone could be detected based on a slight increase in the absorption intensity of the complex simultaneous with an increase in the concentration of CT-DNA. Further investigations were based on the immunomodulatory effect of this complex. Our study demonstrates that Pt(II) complex has an inhibitory effect on the release of proinflammatory cytokines in ConA activated splenocytes as well as $CD3^+$ T cells derived from the spleens of BALB/c mice. Pronounced anti-inflammatory properties of Pt(II) complex may contribute to the confirmed antitumor effect, but also to illuminate the potential therapeutic effects in various inflammatory diseases, like multiple sclerosis, inflammatory bowel disease, Crohn's disease, systemic lupus erythematosus, gout and rheumatoid arthritis among others. Interestingly, significant immunomodulatory effects of Pt(II) complex were also expressed on previously non-activated splenocytes. Increases in cytokine production after incubation of splenocytes in the presence of the Pt(II) complex only insinuates that the complex might affect cytokine production patterns by other splenocytes except T cells. Further research is necessary in order to clarify the exact mechanism of action of this platinum(II) complex.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13332, or from the corresponding author on request.

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ИЗВОД

ДНК/BSA ИНТЕРАКЦИЈЕ И ИМУНОМОДУЛАТОРНИ ЕФЕКТИ ДИНУКЛЕАРНОГ КОМПЛЕКСА ПЛАТИНЕ(II) СА АРОМАТИЧНИМ 1,5-НАФТИРИДИНСКИМ МОСТНИМ ЛИГАНДОМ

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Евалуација интеракција динуклеарног платина(II) комплекса, [*cis*-{$PtCl(NH_3)_2$}$_2$(μ-1,5-nphe)](ClO_4)$_2$ (1,5-nphe је мостни 1,5-нафтиридински лиганд) са ДНК и BSA испитивана је применом UV–Vis и флуоресцентне емисионе спектроскопије. Испитан је и утицај комплекса платине(II) на имунски одговор. Перитонеални спленоцити изоловани су из здравих мишева BALB/c соја и култивисани са конкавалином A (ConA) и платина(II) комплексом. Измерене су концентрације IFN-γ, IL-1β, IL-17, TNF-α и IL-10 из добијених супернатанта и анализиран је фенотип култивисаних спленоцита. Резултати указују на имуномодулаторне ефекте испитиваног платина(II) компекса. Платина(II) комплекс има инхибиторни ефекат на секрецију проинфламаторних цитокина у ConA активираним спленоцитима и $CD3^+$ Т ћелијама.

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