doi: 10.4149/gpb_2018004

The perfusion of cisplatin and cisplatin structural analogues through the isolated rat heart: The effects on coronary flow and cardiodynamic parameters

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Abstract. The therapeutic use of cisplatin for the treatment of solid tumours is associated with organ toxicity. Amongst those, the cardiotoxicity is an occasional but very serious and severe side effect. To prevent or reduce these negative effects, many cisplatin analogues have been synthesized and evaluated in terms of being a less toxic and more effective agent. In present study, we examined the effects of cisplatin and its three analogues in the isolated rat heart to determine whether changes in the structure of the platinum complexes (changing of carrier ligands – ethylenediamine; 1,2-diaminocyclohexane; 2,2':6',2"-terpyridine) can influence their cardiotoxic effects. The results of our research indicate that the introduction of aromatic rings in the structure of the platinum complexes has a negative influence on the heart function. Conversely, the other two examined complexes had less negative effects on heart function compared to cisplatin. Our findings may be of interest for a possible synthetic strategy of introducing a carrier ligand that will exert a less cardiotoxic effect.

Key words: Cardiac function — Cardiotoxicity — Cisplatin — Isolated-perfused heart — Metal toxicity — Platinum(II) complexes

Introduction

Cis-diaminedichloroplatinum(II) (cisplatin – CDDP) is a first generation antitumour agent with a proven clinical efficacy in the treatment of solid tumours. However, drug resistance and numerous side-effects limit its therapeutic use (Giaccone 2000; Frezza et al. 2010). Cisplatin cancer chemotherapy can cause acute vascular events and may be associated with an increased long-term cardiovascular risk, electrocardiographic changes, arrhythmias, myocarditis, cardiomyopathy and congestive heart failure (Pai and Nahata 2000; Yeh et al. 2004; Herrmann et al. 2016). The degree of heart injury in humans during cisplatin chemotherapy highly depends on the administered dose (Demkow et al. 2013; Higgins et al. 2015). The mechanism of cisplatin's action on cancer cells is relatively well-known (damaging DNA, inhibiting DNA synthesis and mitosis, and inducing apoptotic cell death), whilst the mechanisms of cisplatin-induced cardiotoxicity are still not clear (El-Awady et al. 2011; Dasari and Tchounwou 2014). Several experimental and clinical studies suggested that the cardiotoxicity can be direct consequence of a cisplatin-induced increase in the production of reactive oxidative species (ROS) (Al-Majed et al. 2006; Ferroni et al. 2011). To reduce the systemic toxicity of cisplatin, a thousand of platinum complexes and cisplatin

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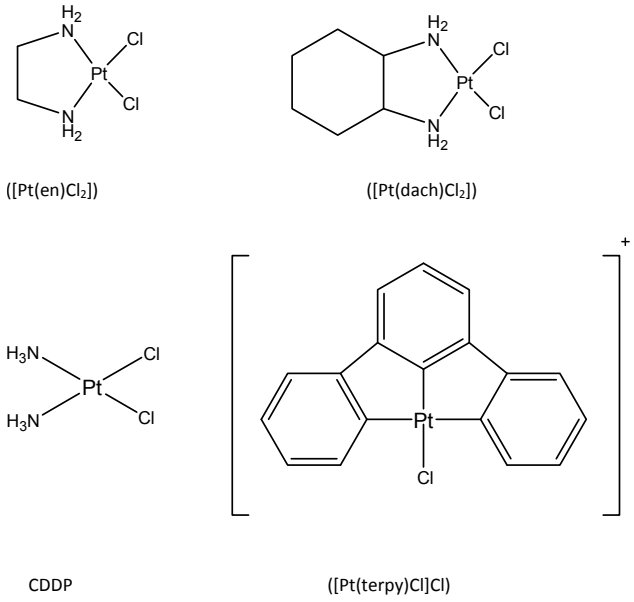

Figure 1. Structures of the examined complexes

analogues have been synthesized and evaluated (Medici et al. 2015). The cisplatin analogues differ in terms of their structures, chemical reactivity, solubility, pharmacokinetics and toxicity (Dasari and Tchounwou 2014). Clinical studies have shown that carboplatin and oxaliplatin had a lower potential to induce systemic toxicity (neurotoxicity, nephrotoxicity, gastrointestinal and metabolic toxicity) (Ozols et al. 2003; Kolomeyevskaya et al. 2015). Therefore, in the present study, we examined and compared the effects of cisplatin (reference compound) and Pt(II) analogues containing ethylenediamine (en), 1,2-diaminocyclohexane (dach) and 2,2':6',2"-terpyridine (terpy) as a carrier ligand on cardiac function and coronary flow in the isolated and perfused rat heart (Figure 1) (Connors et al. 1972; Cleare et al. 1978; Kidani et al. 1978; Ahmadi et al. 2006).

The aim of this experimental study was to determine whether structural changes in the complexes (same valence state, different carrier ligand and same leaving ligand-chloride) can modify the cardiotoxic effects in comparison to cisplatin.

Material and Methods

Isolated rat heart preparation

The hearts were excised from male Wistar albino rats that were aged between ten and twelve weeks (body mass 200–220 g) and were kept in cages (4 animals/cage: 55 cm L × 40 cm W × 20 cm H) with a day/night cycle (12:12). After short-term anaesthesia with ketamine (10 mg/kg) and xylazine (5 mg/kg), the animals were euthanized *via* cervical dislocation (Schedule 1 of the Animals/Scientific Procedures, Act 1986, UK). Following an urgent thoracotomy and a rapid cardiac arrest by superfusion with ice-cold isotonic saline, the hearts were promptly excised and attached to the Langendorff apparatus *via* aortic cannulation, and then they were retrogradely perfused under a constant perfusion pressure of 70 cm H_2O with Krebs-Henseleit solution. The composition of Krebs-Henseleit solution was as follows (in mmol/l): NaCl 118, KCl 4.7, CaCl2 × 2 H_2O 2.5, MgSO4 × 7 H_2O 1.7, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, pyruvate 2, equilibrated with 95% O_2 plus 5% CO_2, and warmed to 37°C (pH 7.4). Immediately after the establishment of automatic operation, a sensor (transducer BS4 73-0184, Experimetria LTD, Budapest, Hungary) was placed into the left ventricle through an incision in the left atrium adjacent to the severed mitral valve to register the pressures.

Experimental groups

The hearts were divided into four main experimental groups and one control group: 1. Control, perfusion with Krebs-Henseleit solution; 2. CDDP, perfusion with cisplatin; 3. $Pt(en)Cl_2$, perfusion with dichloro-(ethylenediamine) platinum(II)]); 4. $Pt(dach)Cl_2$, perfusion with dichloro-(1,2-diaminocyclohexane)platinum(II) ([]); 5. Pt(terpy)Cl]Cl, perfusion with chloro-(2,2':6',2"-terpyridine)platinum(II) chloride dihydrate; *n* =36 rats *per* experimental group.

All experimental groups were divided into three subgroups in accordance with the applied dose of the Platinum(II) complex: 1st subgroup 10^{-5} mol/l, 2nd subgroup 10^{-6} mol/l and 3rd subgroup 10^{-7}mol/l. It was taken into consideration that a single dose of cisplatin (10^{-6} mol/l) can cause cardiotoxicity (Wang et al. 2009). We used that dose, one higher dose (10^{-5} mol/l) and one lower dose to determine whether cardiotoxic effects are dose-dependent.

All tested substances were administered for 30 minutes, which was followed by a washout period of 15 minutes, according to a previously reported protocol (Peric et al. 2012).

All research procedures were carried out in accordance with the European Directive for the welfare of laboratory animals No 86/609/EEC and principles of Good Laboratory Practice (GLP), approved by an Ethical Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

Experimental protocol

All hearts underwent a stabilization period, and during this period, each of the hearts was subjected to a short-term occlusion (20 s) followed by simultaneous bolus injections of 5 mmol/l adenosine (60 µl at a flow of 10 ml/min to elicit maximal coronary flow) to test the coronary vascular reactivity. Coronary flow (CF), as a parameter of coronary circulation, was measured flowmetrically. In all experimental

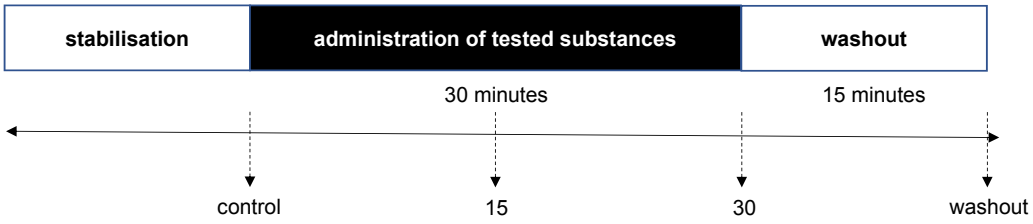

Figure 2. Experimental protocol on an isolated rat heart.

groups, the following parameters of heart function were continuously recorded: maximum rate of left ventricular pressure development ($LV(dP/dt)_{max}$), minimum rate of left ventricular pressure development ($LV(dP/dt)_{min}$), left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), and heart rate (HR).

The end of the stabilization period and the beginning of the experimental period was when the CF was stabilized (three successive measurements of the same value). At the beginning of the experiment, all cardiodynamic parameters and CF were measured, and those values were marked as the baseline value. Then, the perfusion was started with an appropriate concentration of each compound, depending on the group.

We also measured all cardiodynamic parameters and CF at the last minute of the substance application (effect) and at the last minute of the washout period (washout) (Figure 2).

Tissue processing for light microscopy

Tissue was prepared for morphological analysis as described in previous publications. After immersion fixation in 4% neutrally buffered formaldehyde, the tissue was dehydrated and embedded in paraffin and then sectioned in 4-micrometre thick sections and stained with Masson's trichrome.

Morphological changes were described and graded, and the tissue damage score was obtained according to previously established and modified grading systems (Trajkovic et al. 2007). The pathologist who performed the analysis was blinded to the data from the experimental groups.

Substances (chemicals)

All chemicals and cisplatin were obtained from Sigma-Aldrich GmbH, Germany, and were of p.a. grade quality.

Platinum(II) complexes have been synthesized according to described procedures (Annibale et al. 1995; Galanski and Keppler 1995).

Statistical analysis

All values are expressed as the mean ± SD. A Wilcoxon signed-rank test (for the difference between related samples) was used to analyse effects of a substance in the subgroup (baseline *vs.* effect; effect *vs.* washout; baseline *vs.* washout). The Wilcoxon signed-rank test was used in the statistical analysis, and p values less than 0.05 were statistically significant. For the comparison of the complexes' effects amongst the groups, we used an ANOVA, and p values less than 0.05 were statistically significant. For multiple comparisons after the ANOVA, we used a *post hoc* test (Bonferroni when homogeneity of variance was higher than 0.05 and Dunnett T3 when homogeneity of variance was lower than 0.05). The statistical analysis was performed using SPSS 19.0 for Windows.

Results

The effects of cisplatin administration on cardiodynamic parameters and coronary flow

The $LV(dP/dt)_{max}$ was significantly decreased only after administration the highest dose of cisplatin when effect and also washout were compared to baseline (Fig. 3A). The administration of all doses of cisplatin induced a statistically significant decrease in $LV(dP/dt)_{min,}$ when washout was compared to baseline (Fig. 3B, 4B, 5B), while middle and the lowest dose also induced decrease when effect was compared to baseline (Fig. 4B, 5B). All doses of cisplatin induced decrease of LVSP, when washout was compared to baseline (Fig. 3C, 4C, 5C). In addition, the middle and the highest doses induced decrease of LVSP when effect was compared to baseline (Fig. 4C, 3C). Also after administration of the highest dose, decrease of LVSP was observed when effect was compared with washout (Fig. 3C). The administration of the highest dose of cisplatin induced decrease of HR when effect and also washout were compared to baseline (Fig. 3E). On the other hand, middle dose induced decrease when washout was compared to baseline (Fig. 4E), while the lowest dose induced decrease of HR when effect was compared to baseline Fig. 5E). The CF was significantly decreased only after administration the highest dose of cisplatin, when washout was compared to baseline (Fig. 3F). All the observed changes in the cardiodynamic parameters were irreversible, except for a decrease HR after administration of the lowest dose. The LVDP was not significantly changed after the administration of any dose (Fig. 3D, 4D, 5D).

The effect of [Pt(en)Cl_2] administration on cardiodynamic parameters and coronary flow

The administration of the highest and the lowest doses of [Pt(en)Cl_2] induced a statistically significant decrease in LV(dP/dt)$_{min}$ when washout was compared to baseline (Fig. 3B, 5B). The administration of the highest and the lowest doses of [Pt(en)Cl_2] induced a statistically significant decrease of LVSP when washout was compared to baseline (Fig. 3C, 5C). LVSP was also decreased when effect was compared to baseline, after administration of the highest dose (Fig. 3C). On the other hand, the administration of the highest and middle doses of [Pt(en)Cl_2] induced a significant decrease of HR, when effect was compared to baseline (Fig. 3E, 4E), while the middle dose also induced decrease when washout was compared to baseline (Fig. 4E). All observed changes in cardiodynamic parameters were irreversible. The values of LV(dP/dt)$_{max}$ (Fig. 3A, 4A, 5A), LVDP (Fig. 3D, 4D, 5D) and CF (Fig. 3F, 4F, 5F) were not statistically significantly changed during [Pt(en)Cl_2] administration.

The effect of [Pt(dach)Cl_2] administration on cardiodynamic parameters and coronary flow

The administration of all doses of [Pt(dach)Cl_2] induced a significant decrease in LV(dP/dt)$_{min}$ when washout was compared to baseline (Fig. 3B, 4B, 5B). The administration

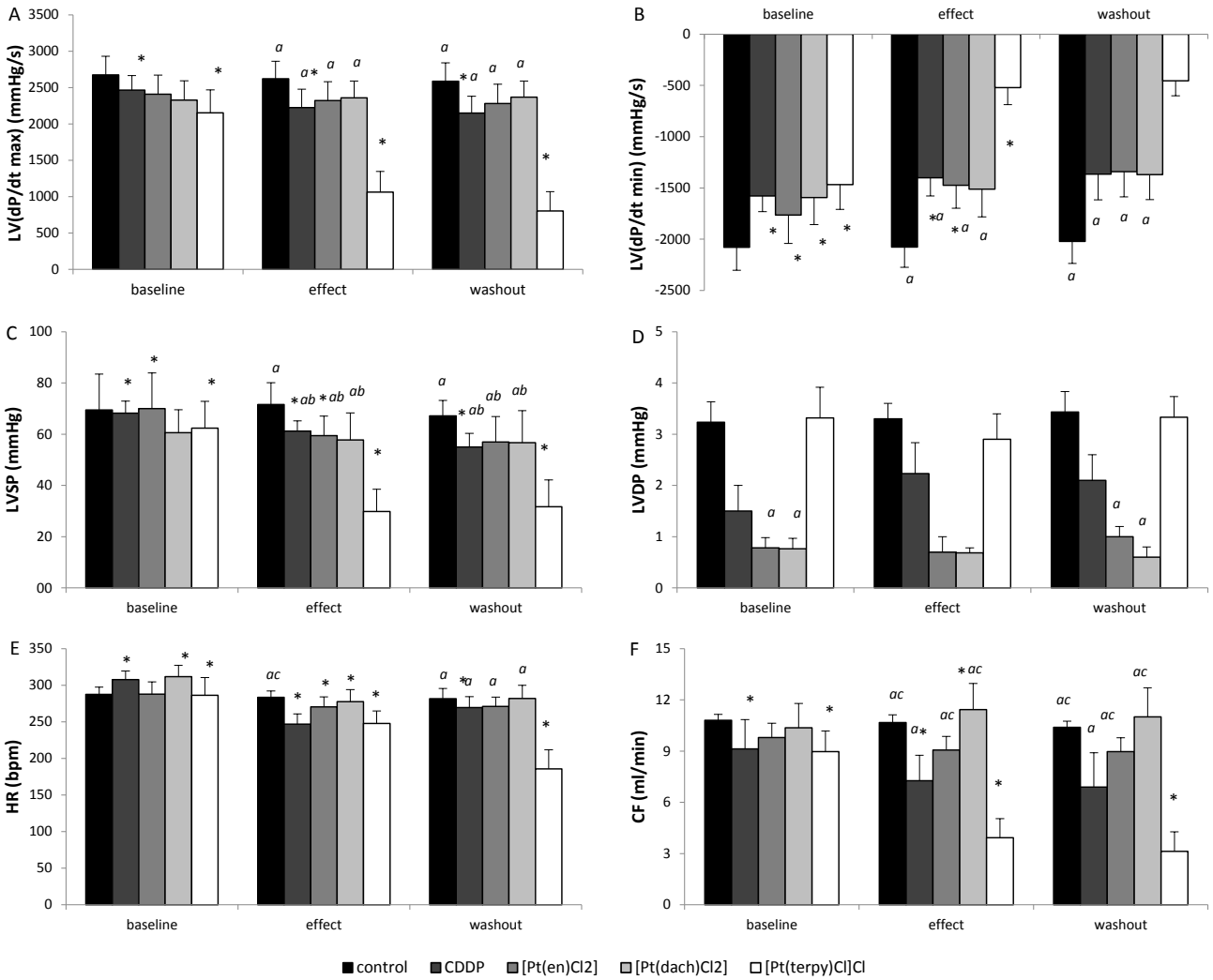

Figure 3. The effects of the highest dose (10^{-5} mol/l) of platinum(II) complexes on cardiodynamic parameters and coronary flow. The results are presented as the mean ± SD (n = 12). The differences within the same group induced by complex: * $p < 0.05$ baseline *vs.* washout. The differences between the groups induced by different complexes are labelled with letters: [a] $p < 0.05$ *vs.* [Pt(terpy)Cl]Cl; [c] $p < 0.05$ *vs.* cisplatin at the respective time point of the experiment.

of middle and the lowest doses induced decrease of LVSP when washout was compared to baseline (Fig. 4C, 5C), while the lowest dose also induced decrease when effect was compared to baseline (Fig. 5C). The administration of the highest and middle doses of [Pt(dach)Cl_2] induced a significant decrease in HR when effect and also washout were compared to baseline (Fig. 3E, 4E). The administration of the highest dose of [Pt(dach)Cl_2] induced increase of CF when effect was compared to baseline (Fig. 3F). All observed changes in cardiodynamic parameters were irreversible. LVDP (Fig. 3D, 4D, 5D) and LV$(dP/dt)_{max}$ (Fig. 3A, 4A, 5A) were not significantly changed after the administration of any dose.

The effect of [Pt(terpy)Cl]Cl administration on cardiodynamic parameters and coronary flow

LV$(dP/dt)_{max}$ was significantly decreased only after the administration of the highest dose of [Pt(terpy)Cl]Cl when all points of interest were compared (Fig. 3A). The administration of [Pt(terpy)Cl]Cl in all doses induced a statistically significant decrease of LV$(dP/dt)_{min}$ when effect was compared to baseline (Fig. 3B, Fig. 4B, Fig. 5B). While the highest and middle doses induced decrease of LV$(dP/dt)_{min}$ when washout was compared to baseline. All doses of [Pt(terpy)Cl]Cl induced decrease of LVSP when washout was compared to baseline (Fig. 3C, 4C, 5C). In addition,

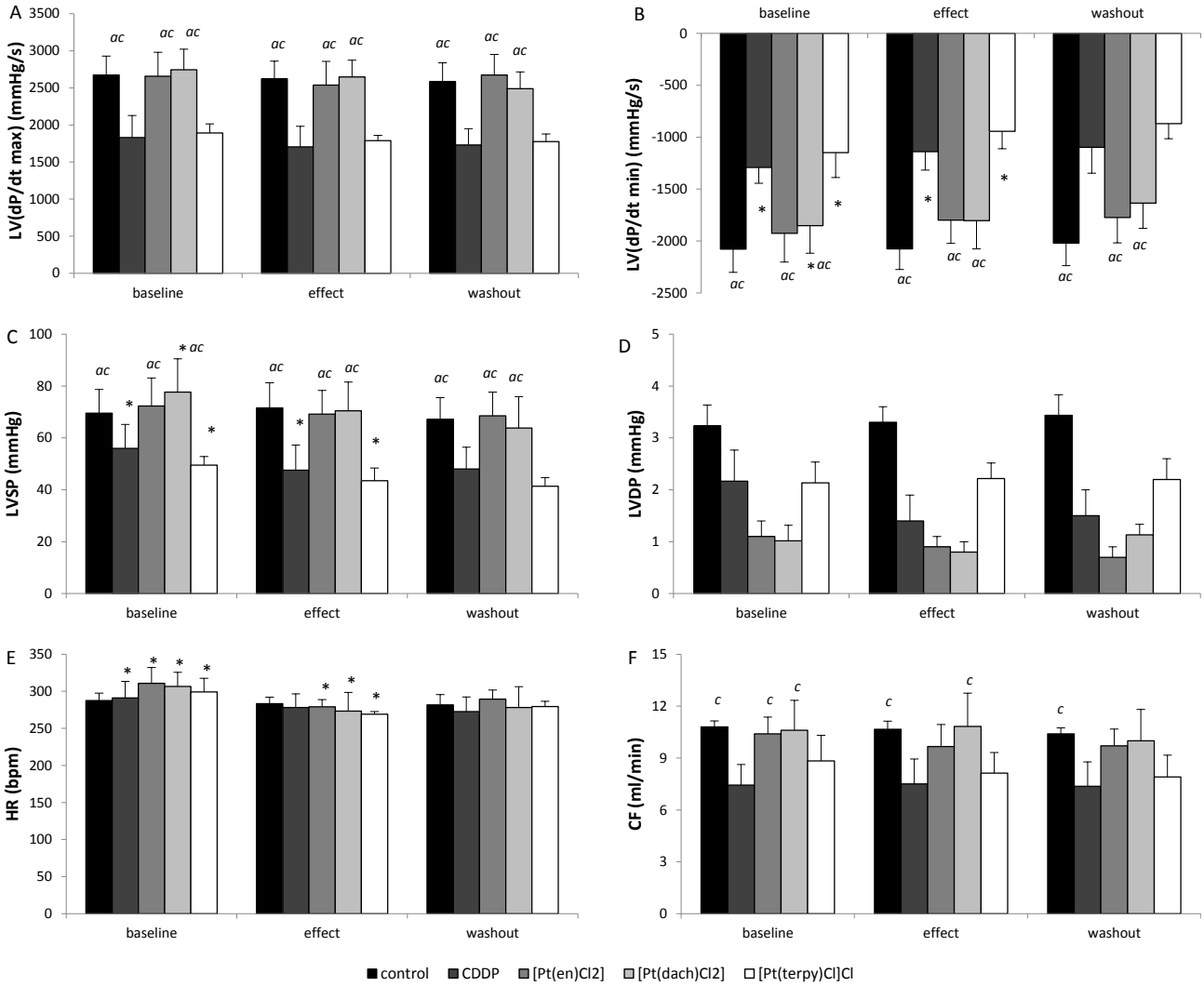

Figure 4. The effects of middle dose (10^{-6} mol/l) of platinum(II) complexes on cardiodynamic parameters and coronary flow. The results are presented as the mean ± SD (n = 12). The differences within the same group induced by complex: * $p < 0.05$ baseline *vs.* washout. The differences between the groups induced by different complexes are labelled with letters: [a] $p < 0.05$ *vs.* [Pt(terpy)Cl]Cl; [c] $p < 0.05$ *vs.* cisplatin at the respective time point of the experiment.

the middle and the highest doses induced decrease of LVSP when effect was compared to baseline (Fig. 4C, 3C). Also after administration of the highest dose, decrease of LVSP was observed when effect was compared with washout (Fig. 3C). HR was significantly decreased after the highest and middle dose when washout and also effect were compared to baseline (Fig. 3E, 4E), while the highest dose also induced decrease when washout was compared to effect (Fig. 3E). CF was significantly decreased only after the administration of the highest dose of [Pt(terpy)Cl]Cl when all points of interest were compared (Fig. 3F). All the observed changes in cardiodynamic parameters were irreversible. The LVDP was not significantly changed after the administration of any dose (Fig. 3D, 4D, 5D).

The effect of Krebs-Henseleit solution administration on cardiodynamic parameters and CF

The administration of only the Krebs-Henseleit solution did not induce any statistically significant changes in the cardiodynamic parameters and coronary flow.

The comparison of the effects of the highest doses of applied complexes on cardiodynamic parameters and CF

[Pt(terpy)Cl]Cl, applied in the highest dose (10^{-5} M), induced a statistically significant higher decrease in LV(dP/dt)$_{max}$, LV(dP/dt)$_{min}$, LVSP and CF compared to the other complexes applied in the same dose as well as the Krebs-

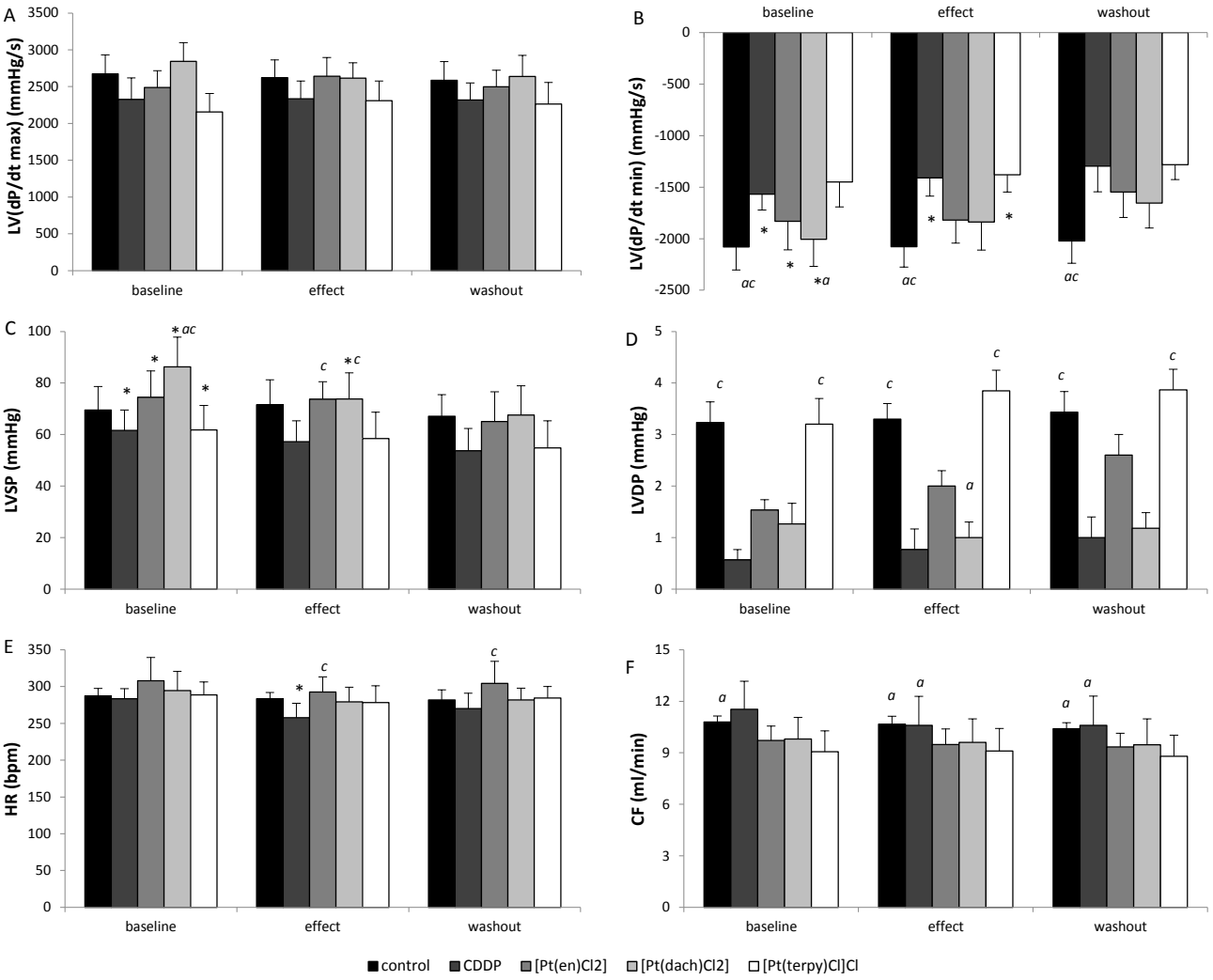

Figure 5. The effects of the lowest dose (10^{-7} mol/l) of platinum(II) complexes on cardiodynamic parameters and coronary flow. The results are presented as the mean ± SD (n = 12). The differences within the same group induced by complex: * $p < 0.05$ baseline *vs.* washout. The differences between the groups induced by different complexes are labelled with letters: [a] $p < 0.05$ *vs.* [Pt(terpy)Cl]Cl; [c] $p < 0.05$ *vs.* cisplatin at the respective time point of the experiment.

Henseleit solution in effect and washout (Fig. 3A, 3B, 3C, 3F). Additionally, the values of LVSP after the administration of the Krebs-Henseleit solution were higher compared to values of LVSP after the administration of all complexes in effect and washout (Fig. 3C). Administration of CDDP induced higher decrease of CF compared to control, $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$ in effect and washout (Fig. 3F). The highest doses of CDDP and [Pt(terpy)Cl]Cl induced statistically significant decrease of HR compared to Krebs-Henseleit solution in effect (Fig. 3E). [Pt(terpy)Cl]Cl induced statistically significant decrease of HR compared to other complexes and control in washout point (Fig. 3E). The baseline values of LVDP amongst the [Pt(terpy)Cl]Cl group and the $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$ groups were significantly different, so the effects of those complexes on LVDP cannot be compared to each other (Fig. 3D). There were no differences between the $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$ groups regarding any of the examined cardiodynamic parameters as well as in coronary flow.

The comparison of the effects of middle doses of applied complexes on cardiodynamic parameters and CF

The baseline of the $LV(dP/dt)_{max}$, $LV(dP/dt)_{min}$ and LVSP in [Pt(terpy)Cl]Cl and cisplatin groups were statistically significant different compared to the $[Pt(en)Cl_2]$, $[Pt(dach)Cl_2]$ and control groups, so we could not compare the effects of those complexes on $LV(dP/dt)_{max}$ (Fig. 4A), $LV(dP/dt)_{min}$ (Fig. 4B) and LVSP (Fig. 4C). In addition, the baseline of CF in cisplatin group was different in comparison with $[Pt(en)Cl_2]$, $[Pt(dach)Cl_2]$ and control groups, so the different effects of those complexes on CF (Fig. 4F) cannot be compared to each other. There were no statistically significant differences in HR between the groups (Fig. 4E).

The comparison of the effects of the lowest doses of applied complexes on cardiodynamic parameters and CF

Cisplatin, at the lowest dose (10^{-7} M), induced a statistically significant decrease in LVSP (Fig. 5C) and HR (Fig. 5E) in effect compared to $[Pt(en)Cl_2]$. As there were significant differences in the basaline values of the other parameters investigated, the effects of individual complexes were not compared to each other.

The effects of Platinum(II) complexes on histopathology of isolated rat heart

The preserved structure of the heart was confirmed by histological analysis in all groups. Cardiomyocytes were of regular morphology, with the proper arrangement of myofibrils and one or two centrally located oval euchromatic nuclei. The morphology of the blood vessels was also preserved. In some samples, focally degenerative changes of cardiomyocytes and vascular changes were observed, which were marked as mild to medium (Table 1). Mild

Table 1. The effects of platinum(II) complexes perfusion on heart tissue of isolated rat heart – tissue damage score for degenerative and vascular changes

Group	Tissue damage score (grade)	Vascular damage score (grade)	Overall tissue damage score
CDDP (10^{-5} mol/l)	1	1	2
CDDP (10^{-6} mol/l)	1	1	2
$[Pt(en)Cl_2]$ (10^{-5} mol/l)	1	3	4
$[Pt(en)Cl_2]$ (10^{-6} mol/l)	1	3	4
$[Pt(dach)Cl_2]$ (10^{-5} mol/l)	1	3	4
$[Pt(dach)Cl_2]$ (10^{-6} mol/l)	1	0	1
[Pt(terpy)Cl]Cl (10^{-5} mol/l)	1	2	3
[Pt(terpy)Cl]Cl (10^{-6} mol/l)	1	2	3
Control	0	0	0

Tissue damage score (grade): 0, Normal finding; 1, single cells with small cytoplasmatic vacuoles, slightly enlarged, and with normal /pyknotic nuclei; 2, >50% cells with mild vacuolization of cytoplasm and nucleoplasm; 3, all cells with pronounced vacuolization of cytoplasm and nucleoplasm, and pycnotic nuclei; 4, pronounced plasmolysis and caryolysis and diffuse infiltration of polymorphonuclear cells (PMNCs) that surround and phagocyte dead cells. Vascular damage score (grade): 0, normal findings; 1, mild dilatation of small blood vessels with no changes in continuation of their walls, but with changes of endothelium; 2, increased blood volume with stasis and hyaline microthrombi; 3, transmural rupture of small number (up to 50%) of blood vessels (mild focal haemorrhagiae diapedesis) associated with no accumulation of PMNCs; 4, complete loss of the basal membrane and endothelial cells of large number of blood vessels (>50%) and high intensity haemorrhagiae diapedesis. Overall tissue damage score is presented as the sum of tissue damage score and vascular damage score.

interstitial and intracellular oedemas along with vacuolization were observed in all groups (Fig. 6A–H), particularly in [Pt(dach)Cl_2] (10^{-5} mol/l) (Fig. 6C). Discrete derangement of myofibrils was noted in [Pt(en)Cl_2] (10^{-5} mol/l) (Fig. 6A), with a partial enlargement of intermyofibrillar spaces on one side of the same cardiomyocyte and a gathering of myofibrils on the other part of sarcoplasm. In almost all groups, "contraction band" necrosis phenomena were clearly visible (Fig. 6A, C–H). Vacuolization and intracellular oedema were followed with karyopiknosis as single cell phenomena in all groups (Fig. 6A–H). Extravasations of red blood cells (haemorrhagiae diapedesis) were most readily observed in the [Pt(en)Cl_2] (10^{-5} mol/l), [Pt(en)Cl_2] (10^{-6} mol/l) and [Pt(dach)Cl_2] (10^{-5} mol/l) groups (Fig. 6A–C). Stasis of fluids and the deposition of fibrine in a form of hyaline microthrombi was encountered only in the [Pt(terpy)Cl]Cl (10^{-5} mol/l) and [Pt(terpy)Cl]Cl (10^{-6} mol/l) groups (Fig. 6G, H).

Discussion

Cisplatin-induced cardiotoxicity, in humans, may occur as an early or late complication during antitumour therapy (Meinardi et al. 2000). In an experimental model of an isolated mouse heart, it has been shown that cisplatin can induce the depression of myocardial contractile function (Ma et al. 2010).

The results of human trials confirmed that administration of 5 mg/kg of cisplatin in total, expressed as weekly dose, resulted in total platinum plasma levels in the range of 1.60–2.60 μg/l (Sunderman et al. 1990). On the other

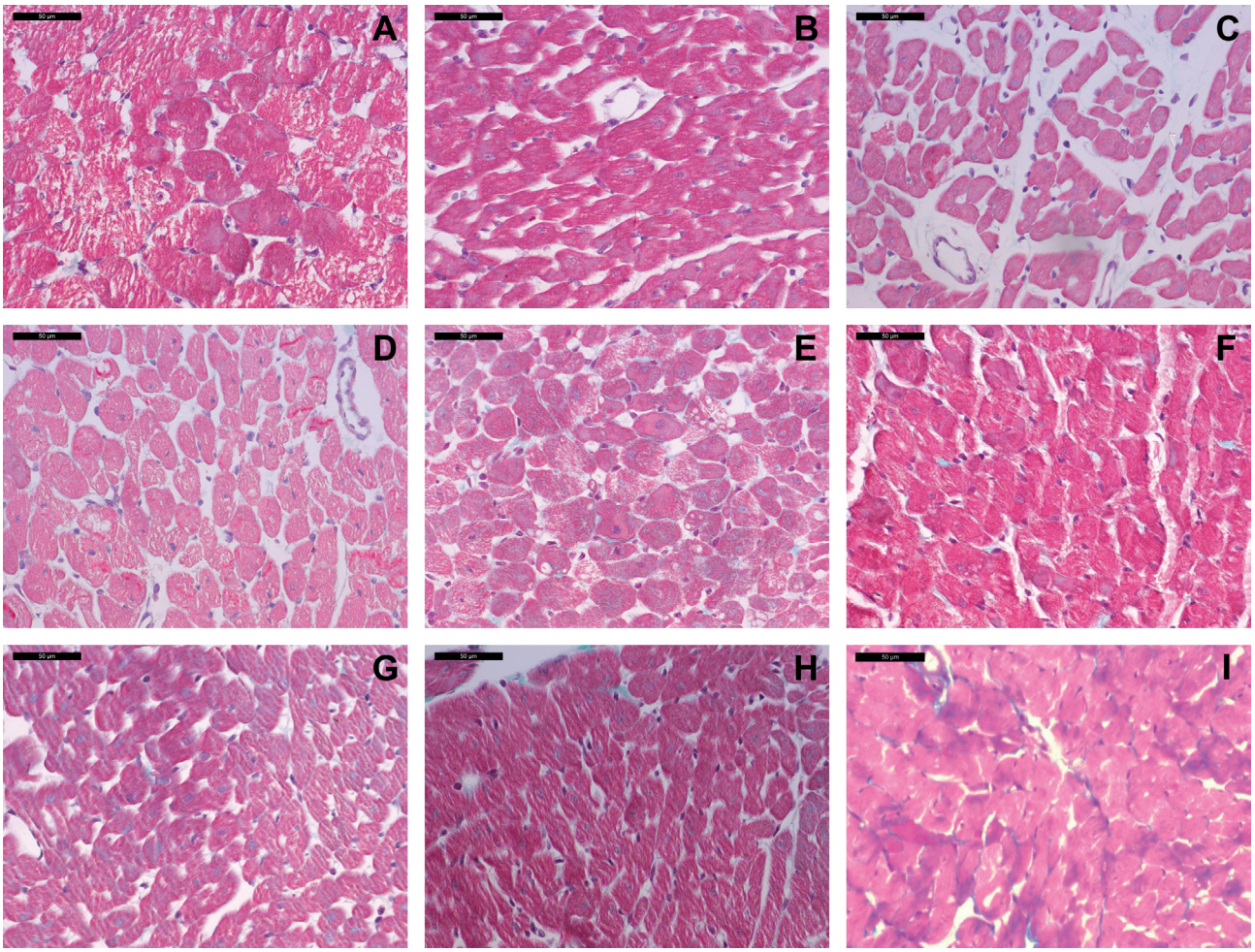

Figure 6. Microscopic evidence of heart toxicity after perfusion of platinum(II) complexes through an isolated rat heart (Masson's trichrome). The effects of [Pt(en)Cl_2] (**A, B**), Pt(dach)Cl_2] (**C, D**) CDDP (**E, F**), [Pt(terpy)Cl]Cl (**G**, **H**) perfusion on heart tissue. **I.** The effects of Krebs-Henseleit solution perfusion on heart tissue. Magnification ×400 (bar = 50 μm). Arrows represented focally observed degenerative changes of cardiomyocytes and vascular changes.

hand, the same concentration of cisplatin, administered as a single dose, in Wistar Albino rats resulted in the mean plasma concentration of total platinum content between 1 and 10 μg/l (Uchino et al. 2005) and tissue levels of cisplatin between 1–40 μg/l (Valentovic et al. 1991). Taking into the consideration that a single dose of cisplatin (7 mg/kg) administered intraperitoneally can induce cardiotoxicity (Wang et al. 2009); we calculated that the appropriate dose for an isolated rat heart is 10^{-6} mol/l. Thus, we examined the effects of this dose, along with one higher and one lower dose.

In the presented study, cisplatin and [Pt(terpy)Cl]Cl showed the greatest impact on myocardial function at the highest (Fig. 3A,B) and middle doses (Fig. 4A,B), compared to other complexes. Nevertheless, comparing the effects of these two complexes at higher dose [Pt(terpy)Cl]Cl had the most depressive effect (Fig. 3A,B). The highest dose induced a decrease in LV$(dP/dt)_{min}$ in all experimental groups (Fig. 3B). Our results for the effects of CDDP on contractility agree with a previous study (Ma et al. 2010). Ma et al. showed that a one-week treatment with cisplatin (10 mg/kg *per* day i.v.) reduced left ventricular developed pressure in isolated C57BL/6 mice hearts retrogradely perfused with Krebs-Henseleit buffer. They demonstrated that cisplatin-induced cardiac dysfunction was associated with mitochondrial membrane depolarization. The changes of contractility, observed in our study, induced by perfusion with the complexes were completely irreversible. The values of LV(dP/dt)max and LV(dP/dt)min were lower after the washout period and related to values recorded at the end of perfusion of applied substances (Fig. 3A, 3B, 4A, 4B, 5A, 5B).

The values of LVSP were affected by perfusion of isolated rat heart with all complexes, but to a different extent. Cisplatin and [Pt(terpy)Cl]Cl induced decrease of LVSP regardless of the applied dose. On the other hand [Pt(dach)Cl_2] induced decrease of LVSP at middle and the lowest doses, and [Pt(en)Cl_2] induced decrease of LVSP at the highest and the lowest doses. All changes were completely irreversible. Our results agree with the findings that cisplatin use can decrease the mean arterial blood pressure (Ma et al. 2010; El-Sawalhi et al. 2014). El-Sawalhi et al. demonstrated that a single dose of cisplatin (7 mg/kg *i.p.*) in Wistar male rats can induce a significant decrease in mean arterial blood pressure and heart rate. These effects are observed five days after cisplatin administration.

All complexes had a great impact on HR at the highest (Fig. 3E) and the middle (Fig. 4E) doses, and only cisplatin induced a decrease in HR at the lowest dose *vs.* baseline (Fig. 5E). The largest reduction in HR was induced by cisplatin and by [Pt(terpy)Cl]Cl compared to control group (Fig. 3E). This result agrees with previously published data (Wang et al. 2009; El-Sawalhi et al. 2014). Wang et al. using the same model as Sawalhi et al, showed that cisplatin induced a significant decrease of HR and LVSP using tail-cuff plethysmographic methods.

Cisplatin-induced bradycardia usually occurs at the very beginning of cisplatin chemotherapy (Altundağ et al. 2001; Dieckmann et al. 2011; Kounis et al. 2016; Kucharz et al. 2016). In our experiment, we found similar trend for HR. The mechanism of cisplatin-induced arrhythmia is unknown, and it remains to be examined.

Our histopathology results, for all experimental groups, have shown focal irregularly arranged myofibrils with a hypercontraction of sarcomeres. Their sarcomeres appeared shorter and occasionally with contraction band necrosis, mostly seen at higher doses (Table 1 and Fig. 6). This phenomenon could potentially be explained by an uncontrolled influx of Ca^{2+} ions, leading to a dissociated contraction of the cardiomyocytes with hypercontraction of the sarcomeres and contraction band necrosis. Accordingly, this could be one reason for the depressive effect on the myocardium.

The highest dose of cisplatin and [Pt(terpy)Cl]Cl induced decrease in CF, which was significant in comparison with effects of the remaining two complexes and the Krebs-Henseleit solution (Fig. 3F). The highest dose of [Pt(dach)Cl_2] induced an increase in CF (Fig. 3F) *vs.* baseline. Other doses of complexes did not induce a significant reduction in CF. The strongest reduction in CF was with [Pt(terpy)Cl]Cl perfusion (65%). The dramatic reduction in CF in the group treated with [Pt(terpy)Cl]Cl at the higher dose could be potentially explained by the formation of hyaline microthrombi that was only observed in this group (Table 1 and Fig. 6G). The formation of hyaline microthrombi is an indicator of disrupted microcirculation and haemostasis (Oehmichen et al. 1986).

Hydrolysis reactions of platinum(II) complexes (platinum ammine halides) depend on a carrier ligand and its voluminosity (Bugarcic et al. 2012; Johnstone et al. 2016). According to the values of the reaction rate constants, it can be concluded that our examined complexes are good nucleophiles. The reactivity of complexes depends on the voluminosity of the carrier ligands. [Pt(dach)Cl_2] has the most voluminous ligand (amongst [Pt(en)Cl_2], [Pt(dach)Cl_2] and [Pt(NH_3)Cl_2]), and it has the slowest substitution reaction rate. Considering that the dach ligand has a positive inductive effect, platinum in [Pt(dach)Cl_2] becomes less electronegative. On the other hand, [Pt(en)Cl_2] has lower positive inductive effect, so it has faster substitution reaction rate than [Pt(dach)Cl_2]. Since CDDP has unsubstituted amino ligands, it has a faster rate of substitution reaction than that of [Pt(dach)Cl_2] and [Pt(en)Cl_2]. The lower reactivity caused a reduced nonspecific interaction with endogenous molecules; therefore, it lowers toxicity. The introduction of pyridine rings, which are π-acceptors, may increase the electrophilicity on the Pt(II) metal centre due to their electron-withdrawing properties. The addition of these aromatic rings can result

in increasing reaction rates for nucleophilic substitution reactions in the case of $[Pt(terpy)Cl]^+$ (Hofmann et al. 2003; Bugarcic et al. 2004; Summa et al. 2006). These structural differences could be the explanation for why perfusion with [Pt(terpy)Cl]Cl has the strongest myocardial depression related to CDDP, $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$. Our histopathological findings showed that there were no permanent changes during perfusion.

In general, we noted that the deleterious effects on heart function and CF are more pronounced with [Pt(terpy)Cl] Cl compared to the other examined complexes. This finding agrees with the known statement that introducing an aromatic ring in a drug's structure can increase toxicity. The two other complexes had shown a less negative effect on heart function compared to cisplatin. In that sense, these findings can be of interest for a possible synthetic strategy for new platinum-based anticancer agents in terms of their carrier ligand structure, mode of coordination and valence state, which can lead to platinum-based drugs with less cardiotoxic potentials.

Acknowledgements. This work was supported by grant No. 175043 and 175007 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Received: June 1, 2017
Final version accepted: January 18, 2018