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Original Article

In vitro effects of platinum compounds on renal cellular respiration in mice

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Abstract: Background: Cisplatin, carboplatin and oxaliplatin are structurally-related compounds, which are commonly used in cancer therapy. Cisplatin (Platinol®) has Boxed Warning stating: "Cumulative renal toxicity associated with PLATINOL is severe", while carboplatin and oxaliplatin are less nephrotoxic. These drugs form platinum adducts with cellular DNA. Their bindings to cellular thiols (e.g., glutathione and metallothionein) are known to contribute to drug resistance while thiol depletion augments platinum toxicity. Methods: Using phosphorescence oxygen analyzer, this study investigated the effects of platinum drugs on renal cellular respiration (mitochondrial O_2 consumption) in the presence and absence of the thiol blocking agent *N*-ethylmaleimide (used here as a model for thiol depletion). Renal cellular ATP was also determined. Kidney fragments from C57BL/6 mice were incubated at 37°C in Krebs-Henseleit buffer (gassed with 95% O_2:5% CO_2) with and without 100 μM platinum drug in the presence and absence of 100 μM *N*-ethylmaleimide for ≤ 6 h. Results: Platinum drugs alone had no effects on cellular respiration ($P \geq 0.143$) or ATP ($P \geq 0.161$). *N*-ethylmaleimide lowered cellular respiration ($P \leq 0.114$) and ATP ($P = 0.008$). The combination of platinum drug and *N*-ethylmaleimide significantly lowered both cellular respiration ($P \leq 0.006$) and ATP ($P \leq 0.003$). Incubations with *N*-ethylmaleimide alone were associated with moderate-to-severe tubular necrosis. Incubations with cisplatin+*N*-ethylmaleimide *vs.* cisplatin alone produced similar severities of tubular necrosis. Tubular derangements were more prominent in carboplatin+*N*-ethylmaleimide *vs.* carboplatin alone and in oxaliplatin+*N*-ethylmaleimide *vs.* oxaliplatin alone. Conclusions: These results demonstrate the adverse events of thiol depletion on platinum-induced nephrotoxicities. The results suggest cellular bioenergetics is a useful surrogate biomarker for assessing drug-induced nephrotoxicities.

Keywords: Mitochondria, cellular respiration, renal tissue, O_2 consumption, cisplatin, carboplatin, oxaliplatin

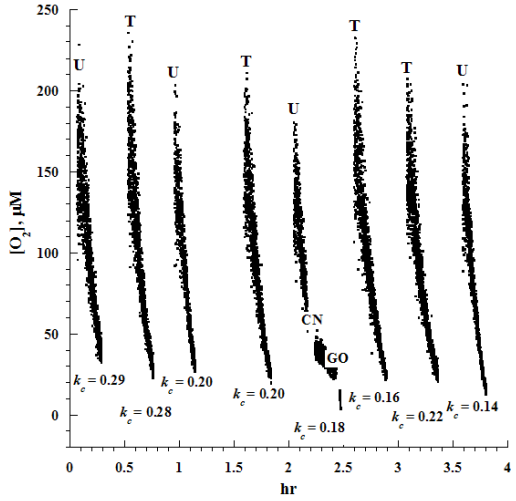
Introduction

Adverse events of medications frequently involve the mitochondria [1]. Drug development, thus, requires *in vitro* testing of candidate compounds for potential induction of mitochondrial dysfunction. This task can be achieved by measuring the effect of drugs on tissue cellular respiration (cellular mitochondrial O_2 consumption; the process of delivery of O_2 and metabolic fuels to the mitochondria, oxidation of reduced metabolic fuels in the mitochondrial respiratory chain and passage of electrons to O_2) [2, 3].

The most commonly used platinum (Pt)-based compounds (cisplatin, carboplatin and oxaliplatin) induce cytotoxicities by binding to the nitrogen atoms on the bases of DNA, forming stable lesions (unidentate and bidentate adducts). This reactivity impairs DNA function and promotes cell death by apoptosis [4-6]. Pt drugs also bind cellular thiols, such as glutathione (GSH) and metallothionein. These reactions limit their availability for cellular DNA and contribute to drug resistance [7-10]. Thiol depletion, thus, increases the adverse events of Pt drug therapy. In one study, DNA platination increased 8-fold when cellular thiols were blocked by *N*-ethylmaleimide (NEM, forms thioether bonds with sulfhydryls) [10]. Clinically, depletion of cellular thiols may result from malnutrition, accumulative Pt dosing and use of alkylating agents [11, 12].

Cisplatin

A



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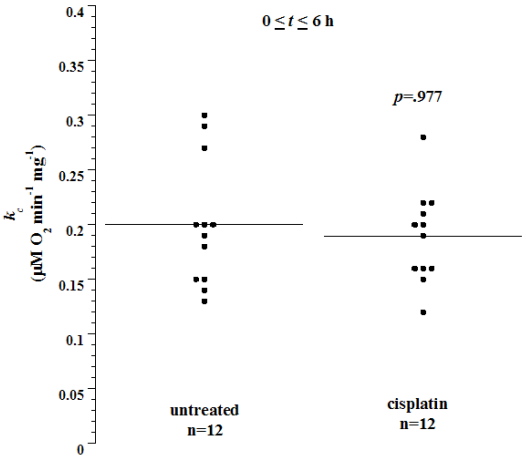
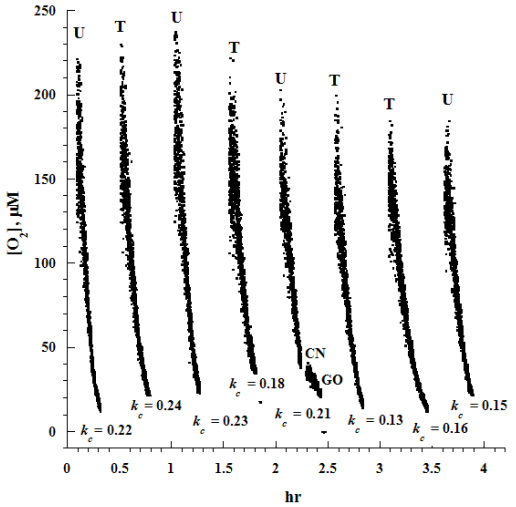


Carboplatin

C



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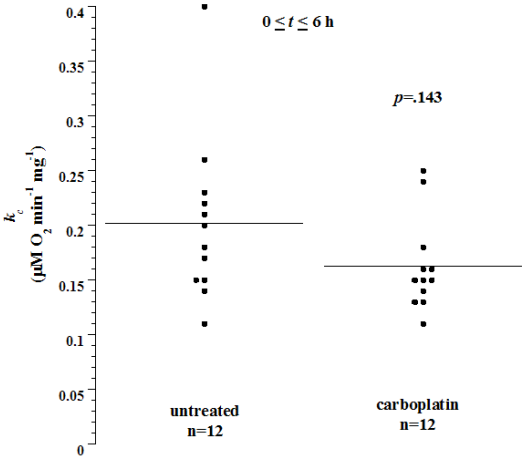
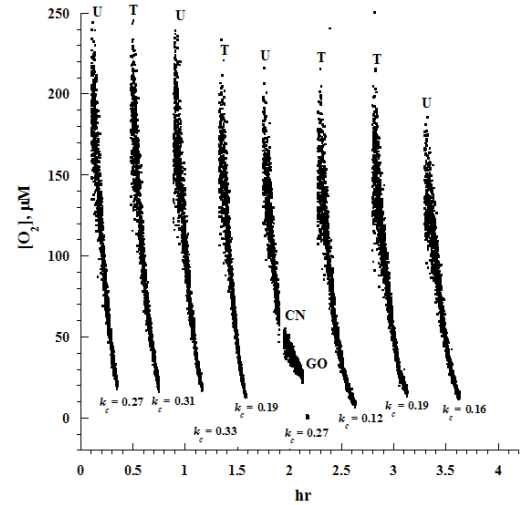


Oxaliplatin

E



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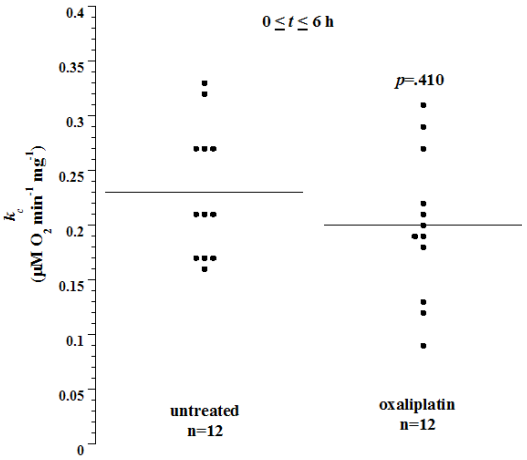


Figure 1. Renal cellular respiration with and without Platinum drugs. Renal specimens were incubated at 37°C in 25 mL KH buffer (gassed with 95% O_2:5% CO_2) with and without 100 μM Pt drugs for up to 6 h. At designated time periods, specimens were removed from the incubation solution, washed with KH buffer and processed for O_2 measurements at 37°C. The rate of respiration (*k,* μM O_2 min^{-1}) was the negative of the slope of [O_2] *vs. t*. The values of k_c (μM O_2 min^{-1} mg^{-1}) are shown at the bottom of each run. The additions of 5 mM NaCN (CN) and 50 μg/mL glucose oxidase (GO; catalyzes the reaction: D-glucose + O_2 → D-glucono-d-lactone + H_2O_2) are also shown. Three separate experiments were performed for each compound; each experiment (one mouse) involved 8 runs (4 untreated and 4 treated). Representative experiments for each Pt drug are shown in Panels A, C and E. Summaries of all results are shown in Panels B, D and F (lines = means). U, untreated; T, treated. The incubation time for all runs was $0 \leq t \leq 6$ h.

The reactions of Pt drugs with cellular thiols are diverse. For example, the second-order rate constants for Pt binding to GSH and metallothionein are much higher with cisplatin and oxaliplatin than carboplatin [9]. This difference is due to the chemical and physical influences of ligands occupying the Pt coordination spheres [13].

It is unknown, however, whether carboplatin toxicity is less sensitive to thiol depletion.

Cisplatin [cis-diamminedichloroplatinum (II)], the first-in-class, is administered intravenously at 20-120 mg/m^2; its maximum free plasma concentration (C_{max}) is 5 to 25 μM and $t_{1/2}$ about 30 min [14]. In high chloride solutions (e.g., the Krebs-Henseleit buffer used in this study), it mostly exists in the neutral form, cis-$Pt(NH_3)_2Cl_2$. At low chloride concentration (inside the cell), the drug produces highly reactive species, such as cis-$[Pt(NH_3)(H_2O)Cl]^+$, cis-$[Pt(NH_3)_2(H_2O)_2]^{+2}$, cis-$[Pt(NH_3)(OH)Cl]$, cis-$[Pt(NH_3)_2(OH)(H_2O)]^{+1}$, and cis-$[Pt(NH_3)_2(OH)_2]$. Moreover, in the presence of carbonate (Krebs-Henseleit buffer), carbonato and bicarbonato derivatives are also formed. All these species, however, interact with cellular DNA and produce cytotoxicities, including nephrotoxicities [4-6, 14, 15].

Carboplatin [cis-diammine (1, 1-cyclobutanedicarboxylato)-platinum (II)] and oxaliplatin [trans-1-diaminocyclohexane oxalatoplatinum] differ from cisplatin with respect to the ligands occupying the Pt coordination spheres. These newer drugs have diverse antitumor activities and adverse events (e.g., prominent myelosuppression) [15]. The plasma C_{max} of carboplatin following intravenous doses of 80-175 mg/m^2 is 31.3 ± 8.0 μM, with a $t_{1/2}$ of 49.1 ± 5.0 min. At a dose of 540 mg/m^2, the C_{max} is 216 μM [15]. The plasma C_{max} of oxaliplatin following standard intravenous dosing of 130 mg/m^2 is 3.8 to 12.1 μM [16].

The effects of Pt compounds on Jurkat cells cellular respiration have been studied [17-19]. More recently, *in vitro* preparations of murine tissue have been developed to fit performing these measurements on viable renal tissue [20-23]. This study employed the same approach to investigate whether Pt drugs impair renal cellular respiration and structure. The effects of blocking renal cellular thiols by *N*-ethylmaleimide were also investigated.

Materials and methods

Reagents

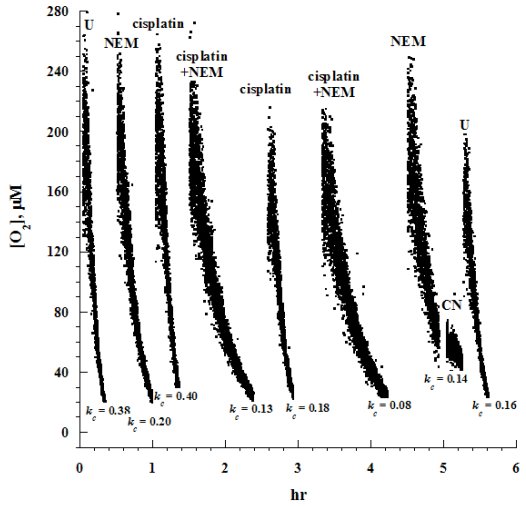
Cisplatin (molecular weight (*m.w)* 300.05, 1.0 mg/mL or 3.3 mM in 154 mM NaCl) was purchased from (Haarlem, Netherlands). Carboplatin (*m.w.* 371.25, 10 mg/mL or 26.9 mM) was purchased was purchased from Actavis (Dublin, Ireland). Oxaliplatin (*m.w.* 397.3, 5 mg/mL or 12.5 mM in tartaric acid, NaOH and H_2O) was purchased as from Hospira UK limited (Queensway, United Kingdom). Palldium (II) complex (Pd) of *meso*-tetra-(4-sulfonatophenyl)-tetrabenzoporphyrin (Pd phosphor) was purchased from Porphyrin Products (Logan, UT). Complete® protease inhibitor cocktail was purchased from Roche Applied Science (Indianapolis, IN). *N*-ethylmaleimide (NEM, forms thioether bonds with sulfhydryls) and the remaining reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Mice

C57BL/6 mice were used in this study. The mice were housed in rooms maintained at 22°C with 60% relative humidity and a 12-h light/dark cycle. They had ad libitum access to standard rodent chow and filtered water. The use of laboratory mice for this study was approved by the Animal Research Ethics Committee at the College of Medicine and Health Sciences, UAE University (Protocol No. A29-13: *In vitro* assessment of the effects of

Cisplatin

A



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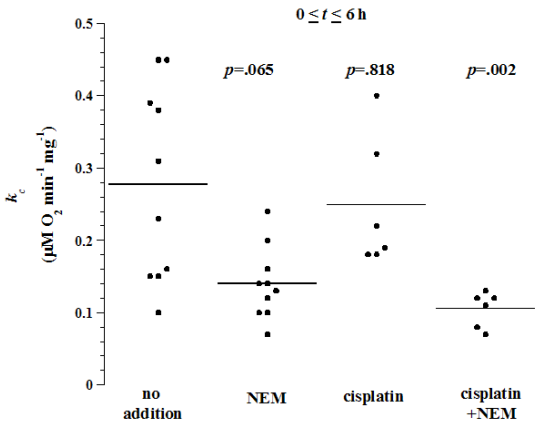
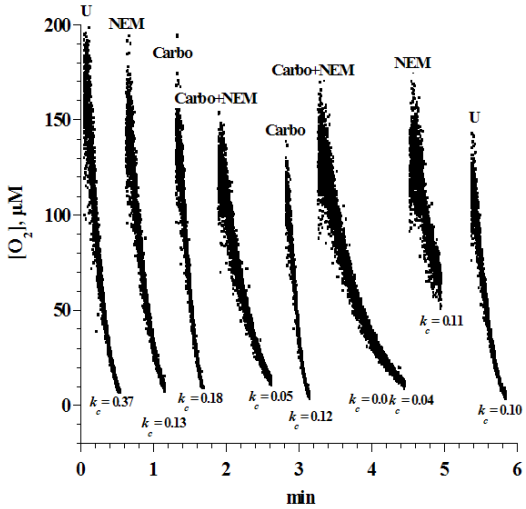


Carboplatin

C



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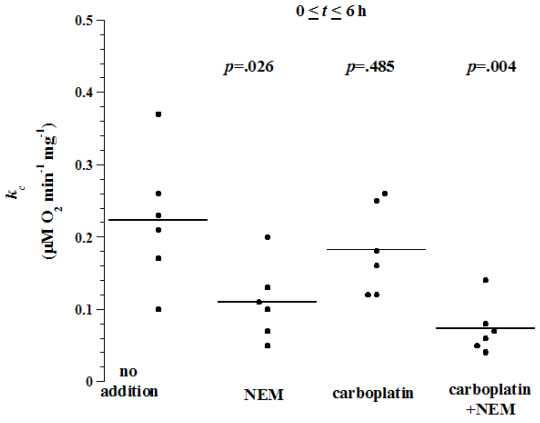
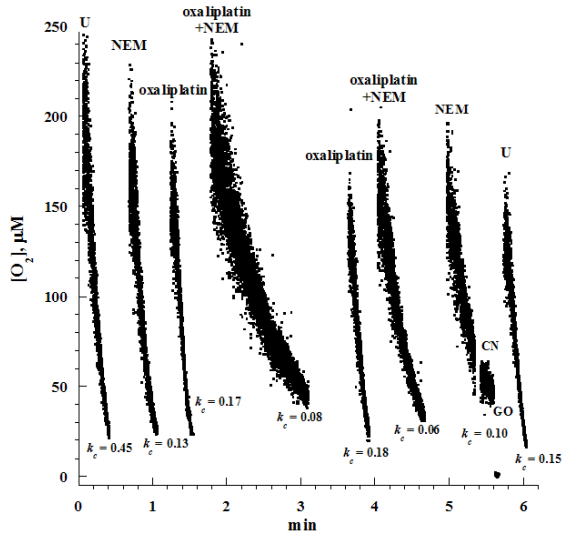


Oxaliplatin

E



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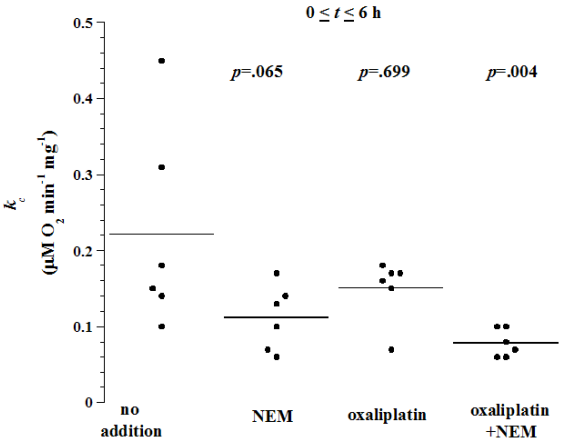


Figure 2. Renal cellular respiration with Platinum drugs and *N*-ethylmaleimide (NEM). Renal specimens were incubated at 37°C in 25 mL KH buffer (gassed with 95% O_2:5% CO_2) with and without 100 μM NEM and 100 μM Pt drugs for up to 6 h. At designated time periods, specimens were removed from the incubation solution, washed with KH buffer and processed for O_2 measurements at 37°C. Three separate experiments were performed for each compound; each experiment (one mouse) involved 8 runs (2 untreated, 2 NEM alone, 2 Pt drug alone, and 2 Pt drug plus NEM). Representative experiments for each Pt drug are shown in Panels A, C and E. Summaries of all results are shown in Panels B, D and F (lines = means). The rates of respiration (k_c, μM O_2 min^{-1} mg^{-1}) are shown at the bottom of each run. The additions of 5 mM CN and 50 mg/mL glucose oxidase (GO) are shown. U, untreated; Carbo, carboplatin. The incubation time for all runs was $0 \leq t \leq 6$ h.

nephrotoxic drugs and toxins on renal cellular respiration in mice).

Renal specimens

Mice were anesthetized by sevoflurane inhalation (10 μL/g). The kidneys were then removed and *immediately* immersed in ice-cold *modified* Krebs-Henseleit (KH) buffer (115 mM NaCl, 25 mM $NaHCO_3$, 1.23 mM NaH_2PO_4, 1.2 mM Na_2SO_4, 5.9 mM KCl, 1.0 mM EDTA, 1.18 mM $MgCl_2$, 10 mM glucose, and 0.5 μL/mL Complete® protease inhibitor cocktail, pH 7.5) gassed with 95% O_2:5% CO_2 as previously described [20-23]. Specimens (cortical slices of 18 to 40 mg each) were excised using a sharp scissor (Moria Vannas Wolg Spring, cat. # ST15024-10). The fragments were incubated at 37°C in 25 mL Krebs-Henseleit buffer (115 mM NaCl, 25 mM $NaHCO_3$, 1.23 mM NaH_2PO_4, 1.2 mM Na_2SO_4, 5.9 mM KCl, 1.25 mM $CaCl_2$, 1.18 mM $MgCl_2$, and 10 mM glucose, pH 7.5) supplemented with 0.5 μL/mL Complete® protease inhibitor cocktail and intermittently gassed with 95% O_2:5% CO_2. The incubation continued with and without additions (Pt compound alone or plus NEM) for up to 6 h. At designated time periods, specimens were removed from the incubation, rinsed with KH buffer and processed for measuring cellular respiration at 37°C in 1-mL sealed vials as previously described and discussed below [20-26].

Histopathology

Renal tissue was fixed in 10% buffered formalin, dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in paraffin. Three-micrometer sections were prepared from paraffin blocks and stained with hematoxylin and eosin (H&E).

Cellular respiration

Briefly, phosphorescence O_2 analyzer was used to monitor O_2 consumption at 37°C as a function of time. $^{24}O_2$ detection was with the aid of Pd phosphor (absorption at 625 nm and phosphorescence at 800 nm) [25]. Samples were exposed to 600 per min light flashes from a pulsed light-emitting diode array peaked at 625 nm. Emitted phosphorescent light was filtered at 800 nm and detected by a Hamamatsu photomultiplier tube. Amplified phosphorescence decays were digitized by a 20-MHz A/D converter (Computer Boards, Inc., Mansfield, MA). A program was developed using Microsoft Visual Basic 6, Microsoft Access Database 2007, and Universal Library components (Universal Library for Measurements Computing Devices; http://www.mccdaq.com/daq-software/universal-library.aspx) [26]. The phosphorescence decay rate ($1/\tau$) was linear with dissolved O_2: $1/\tau = 1/\tau^o + k_q[O_2]$, where $1/\tau$ = the phosphorescence decay rate in the presence of O_2, $1/\tau^o$ = the phosphorescence decay rate in the absence of O_2, and k_q = the second-order O_2 quenching rate constant in $s^{-1} \cdot \mu M^{-1}$ [25]. Oxygen concentration was calibrated using the "glucose/glucose oxidase system" as previously described [20, 24-26].

Cellular ATP

Renal tissue was homogenized in 2% trichloroacetic acid and the supernatants were neutralized with 100 mM Tris-acetate, 2 mM ethylenediaminetetraacetic acid (pH 7.75). ATP was determined using the Enliten ATP Assay System (Bioluminescence Detection Kit and Glomax Luminometer, Promega, Madison, WI) [21, 23].

Statistical analysis

Data were analyzed on SPSS statistical package (version 19), using the nonparametric (2 independent samples) Mann-Whitney test. $P < 0.05$ was considered significant.

Results

Renal cellular respiration (renal tissue O_2 consumption) without any drug addition

Renal cellular respiration was first investigated without any addition. Cellular respiration was

Table 1. Summary of results of renal cellular respiration

		Renal Cellular Respiration (k_c, μM O_2 min^{-1} mg^{-1})	*P*
Cisplatin	Untreated	0.20 ± 0.06	*
	Cisplatin	0.19 ± 0.04	0.977
	Untreated	0.28 ± 0.13	*
	Cisplatin	0.25 ± 0.09	0.818
	NEM alone	0.14 ± 0.05	0.065
	Cisplatin + NEM	0.11 ± 0.02	0.002
Carboplatin	Untreated	0.20 ± 0.08	*
	Carboplatin	0.16 ± 0.04	0.143
	Untreated	0.22 ± 0.09	*
	Carboplatin	0.18 ± 0.06	0.485
	NEM alone	0.11 ± 0.05	0.026
	carboplatin + NEM	0.07 ± 0.04	0.004
Oxaliplatin	Untreated	0.23 ± 0.06	*
	Oxaliplatin	0.20 ± 0.07	0.410
	Untreated	0.22 ± 0.13	*
	Oxaliplatin	0.15 ± 0.04	0.486
	NEM alone	0.11 ± 0.04	0.114
	Oxaliplatin + NEM	0.08 ± 0.02	0.006

Three separate experiments (12 runs involving 3 mice) were performed for each condition. Values are mean ± SD. Drug exposure ≤ 6 h; * = Reference.

Table 2. Summary of results of renal cellular ATP

	Renal Cellular ATP (pmol mg^{-1})	*P*
Untreated	59 ± 21	*
NEM	33 ± 16	0.008
Cisplatin	60 ± 33	0.972
Cisplatin + NEM	27 ± 12	<0.001
Carboplatin	56 ± 19	0.750
Carboplatin + NEM	29 ± 17	0.003
Oxaliplatin	46 ± 15	0.161
Oxaliplatin + NEM	27 ± 9	<0.001

Eight separate experiments were performed (n = 13 untreated specimens; n = 8 treated specimens per condition). Values are mean ± SD. Drug exposure 3 h; * = Reference.

measured at 37°C in glass vials sealed from air and contained a renal specimen and glucose as a respiratory substrate. O_2 concentration then declined linearly with time (zero-order kinetics). The rate (*k*, in μM O_2 min^{-1}) was set as the negative of the slope $d[O_2]/dt$; the value of *k* was divided by the specimen weight (k_c, in μM O_2 $min^{-1}mg^{-1}$). Cyanide (a specific inhibitor of cytochrome oxidase) halted O_2 consumption, confirming the oxidation occurred in the mitochondrial respiratory chain. The addition of glucose oxidase (catalyzes: D-glucose + O_2 → D-glucono-d-lactone + H_2O_2) depleted remaining O_2 in the solution. Examples of O_2 runs are shown in **Figures 1**, **2**.

Six separate experiments (27 runs involving six mice) involving incubations at 37°C without any addition for $0 \le t \le 6$ h were firstly performed. The values of k_c (mean ± SD) were 0.18 ± 0.04 μM O_2 min^{-1} mg^{-1} (coefficient of variation, CV = 22%). These results demonstrated the renal tissue was reasonably stable *in vitro* for at least 6 h.

Renal cellular respiration in the presence of Pt compounds

The effects of cisplatin on renal cellular respiration were then investigated. Cisplatin was tested at 100 μM, a concentration that was about 4-fold higher than typical C_{max} of the drug [14]. Three separate experiments (12 runs involving 3 mice) were performed. A representative experiment is shown in **Figure 1A** and summary of all results in **Figure 1B** and **Table 1**. The rate of respiration (k_c, in μM O_2 $min^{-1}mg^{-1}$) without addition was 0.20 ± 0.06 (CV = 30%, $t \le 216$ min) and with the addition of cisplatin was 0.19 ± 0.04 (CV = 21%, $t \le 183$ min, P = 0.977). Thus, cisplatin at this high dose had no effect on renal cellular mitochondrial O_2 consumption.

For carboplatin, three separate experiments (12 runs involving 3 mice) were also performed. Representative experiment is shown in **Figure 1C** and summary of all results is in **Figure 1D** and **Table 1**. The rate of respiration (k_c, in μM O_2 min^{-1} mg^{-1}) without addition was 0.20 ± 0.08 (CV = 40%, $t \le 290$ min) and with the addition of carboplatin 0.16 ± 0.04 (CV = 25%, $t \le 275$ min, *P* = 0.143). Thus, carboplatin had no effect on renal cellular respiration.

Three separate experiments (12 runs involving 3 mice) were also performed for oxaliplatin. Representative experiment is shown in **Figure 1E** and summary of all results is in **Figure 1F**

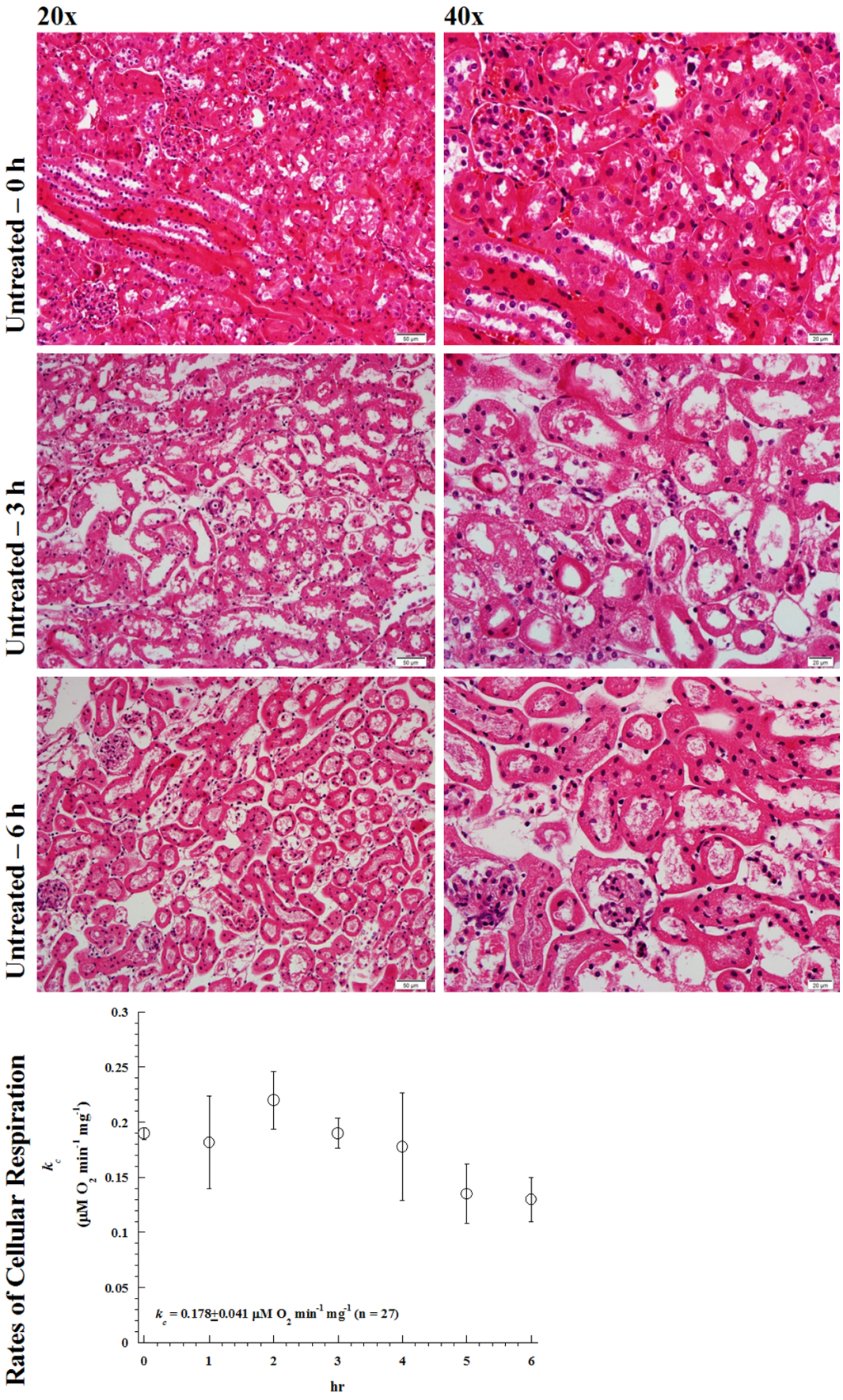

20x
40x
Untreated – 0 h
Untreated – 3 h
Untreated – 6 h
Rates of Cellular Respiration
k_c (μM O_2 min^{-1} mg^{-1})
0.3
0.25
0.2
0.15
0.1
0.05
0
k_c = 0.178±0.041 μM O_2 min^{-1} mg^{-1} (n = 27)
0
1
2
3
4
5
6
hr

Figure 3. Renal histology and cellular respiration without additions.

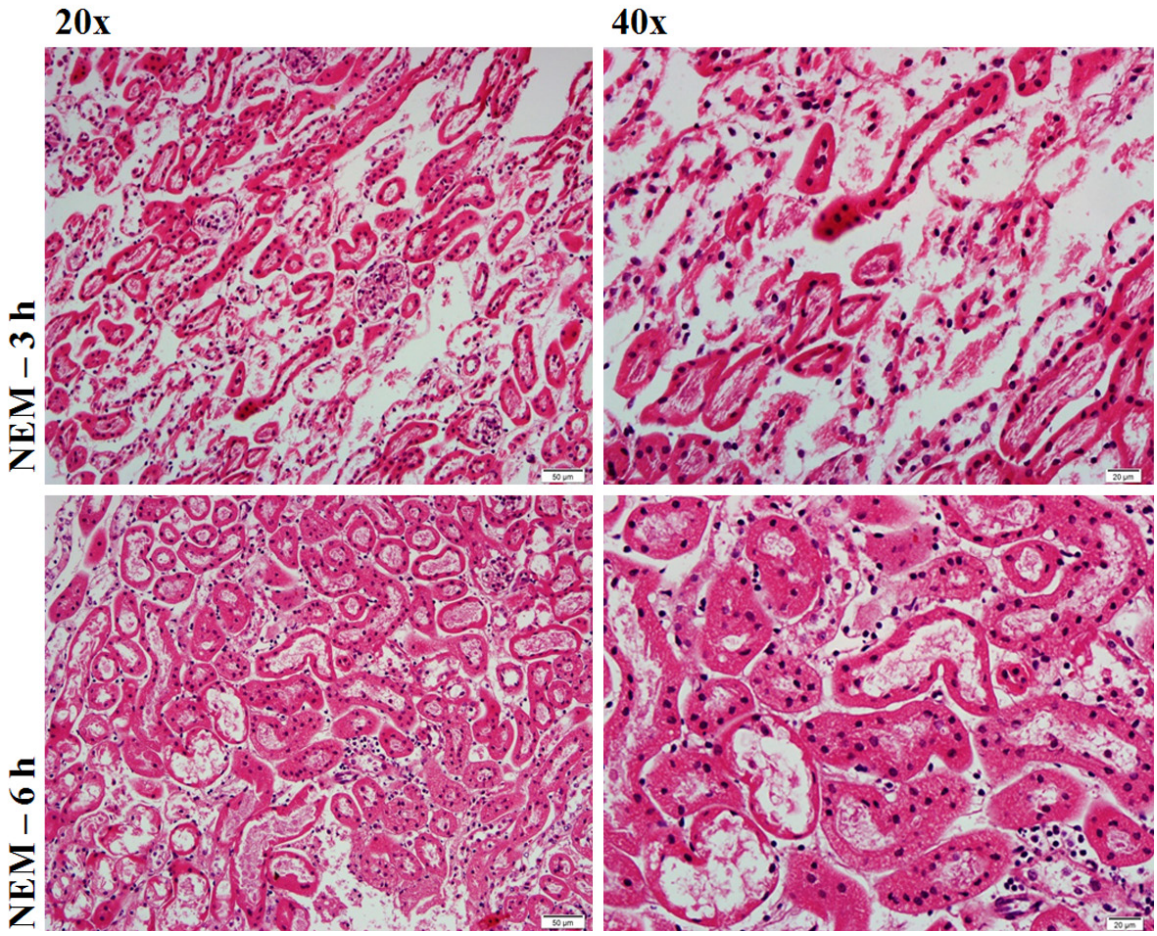


Figure 4. Renal histology with NEM.

and **Table 1**. The rate of respiration (k_c, in µM O_2 min^{-1} mg^{-1}) without addition was 0.23 ± 0.06 (CV = 26%, $t \leq 300$ min) and with the addition of oxaliplatin 0.20 ± 0.07 (CV = 35%, $t \leq 275$ min, P = 0.410). Thus, oxaliplatin had no effect on renal cellular respiration.

Renal cellular respiration with Pt compounds plus NEM

The effect of cellular thiol depletion on Pt-induced nephrotoxicity was then investigated. Three separate experiments (12 runs involving 3 mice for each Pt compound) were performed. Representative experiments and summary of all results are shown in **Figure 2**. For cisplatin (**Figure 2A**, **2B**), the rate of respiration (µM O_2 min^{-1} mg^{-1}) without addition was 0.28 ± 0.13, with 100 µM NEM alone 0.14 ± 0.05 (P = 0.065), with 100 µM cisplatin alone 0.25 ± 0.09 (P = 0.818), and with cisplatin + NEM 0.11 ± 0.02 (P = 0.002).

For carboplatin (**Figure 2C**, **2D**), the rate without addition was 0.22 ± 0.09, with NEM alone 0.11 ± 0.05 (P = 0.026), with carboplatin alone 0.18 ± 0.06 (P = 0.485), and with carboplatin + NEM 0.07 ± 0.04 (P = 0.004).

For oxaliplatin (**Figure 2E**, **2F**), the rate without addition was 0.22 ± 0.13, with NEM alone 0.11 ± 0.04 (P = 0.114), with oxaliplatin alone was 0.15 ± 0.04 (P = 0.486), and with oxaliplatin + NEM 0.08 ± 0.02 (P = 0.006). Thus, for the three studied Pt compounds, respiration was lower with thiol depletion.

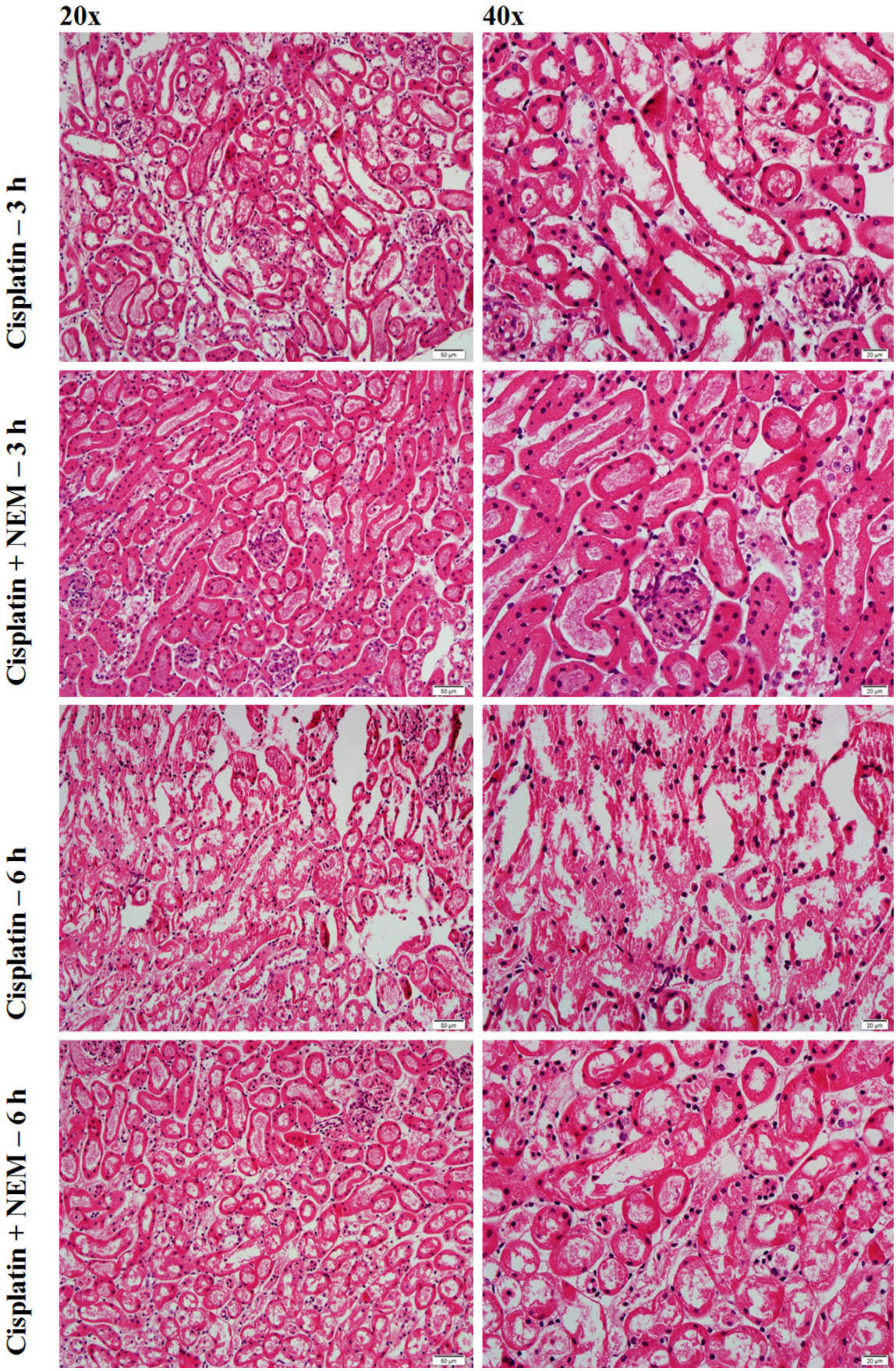

20x
40x
Cisplatin – 3 h
Cisplatin + NEM – 3 h
Cisplatin – 6 h
Cisplatin + NEM – 6 h

Figure 5. Renal histology with cisplatin ± NEM.

Renal cellular ATP

Effects of Pt compounds (with and without NEM) on cellular ATP are shown in **Table 2**; eight separate experiments were performed (8 mice, 13 untreated specimens and 8 treated specimens for each condition). The incubation conditions were as described for cellular respiration; the incubation period was only 3 h. Pt compounds had no effects on cellular ATP ($P \geq 0.161$). NEM alone significantly decreased cellular ATP ($P = 0.008$). Pt compounds plus NEM resulted in further decreases in cellular ATP ($P \leq 0.003$).

Renal histopathology

Figure 3 shows H&E stained renal sections at 0, 3 and 6 h after tissue procurement without additions (untreated specimens). At 0 h (immediately after tissue collection), the glomeruli are unremarkable, the interstitium is indiscernible, and the tubules are back-to-back lined by intact epithelium with preserved brush borders. Based on morphology, there is no evidence of pathologic changes. At 3 h, the glomeruli remain intact. The interstitium is visible and show mild edema. The tubular epithelium reveals mild injury in the form of loss of epithelial brush borders, and flattening of epithelial cells and luminal debris. These findings are consistent with mild acute tubular necrosis (ATN). At 6 h, the glomeruli are unremarkable. The interstitium is appreciable and demonstrate mild edema. The tubules show mild to moderate tubular injury depicted via loss of tubular brush border, attenuation and flattening of tubular epithelium, epithelial sloughing into lumens and luminal debris. These findings are consistent with mild to moderate ATN. Thus, morphology shows reasonable stability of the renal structure at 3 hours with deteriorations at 6 h. Consistently, cellular respiration in untreated specimens incubated for $t \geq 5$ h was significantly less than that for $t \leq 4$ h ($P = 0.001$; **Figure 3**, lower Panel; six separate experiments involving 27 runs).

Figure 4 shows renal histology at 3 and 6 h with NEM alone. The glomeruli are intact. The interstitium show moderate edema. The tubules show moderate to severe tubular injury displayed by loss of tubular brush border, attenuation and flattening of tubular epithelium, epithelial sloughing into lumens and luminal debris. These findings are consistent with moderate (at 3 h) and severe (at 6 h) ATN. Consistently, cellular respiration and ATP with NEM alone ($0 \leq t \leq 6$ h) were significantly less than for untreated specimens (**Tables 1**, **2**).

Figure 5 shows renal histology at 3 and 6 h with cisplatin ± NEM. For cisplatin alone at 3 h, the glomeruli are intact. The interstitium reveals mild edema. The tubules show mild injury displayed by loss of tubular brush border, attenuation and flattening of tubular epithelium, and luminal debris. Findings are consistent with mild ATN (similar to untreated at 3 h). Similar morphological findings are identified with cisplatin + NEM at 3 h. These findings, however, are more prominent at 6 h with and without NEM, revealing moderate-to-severe ATN.

Figure 6 shows renal histology at 3 and 6 h with carboplatin ± NEM. For carboplatin alone at 3 h, the glomeruli are intact. The interstitium reveals mild edema. The tubules show loss of brush border, attenuated flattened epithelium and luminal debris. These findings are in keeping with mild ATN (similar to untreated at 3 h). These findings are more prominent with carboplatin + NEM at 3 h (moderate ATN). The findings are also pronounced at 6 h, especially with NEM, revealing moderate-to-severe ATN.

Figure 7 shows renal histology at 3 and 6 h with oxaliplatin ± NEM. For oxaliplatin alone at 3 h, the glomeruli are intact. The interstitium reveals moderate edema. The tubules show loss of brush border, single cell necrosis, attenuated flattened epithelium and luminal debris, featuring moderate ATN. These findings are similar to oxaliplatin + NEM at 3 h (moderate ATN). At 6 h, the findings with oxaliplatin alone reveal moderate ATN and with oxaliplatin + NEM severe ATN.

Discussion

This study investigated renal toxicities of Pt compounds, using cellular bioenergetics and histopathology as biomarkers. A highly sensitive phosphorescent O_2 probe [palladium II-meso-tetra-(4-sulfonatophenyl)-tetrabenzopor-

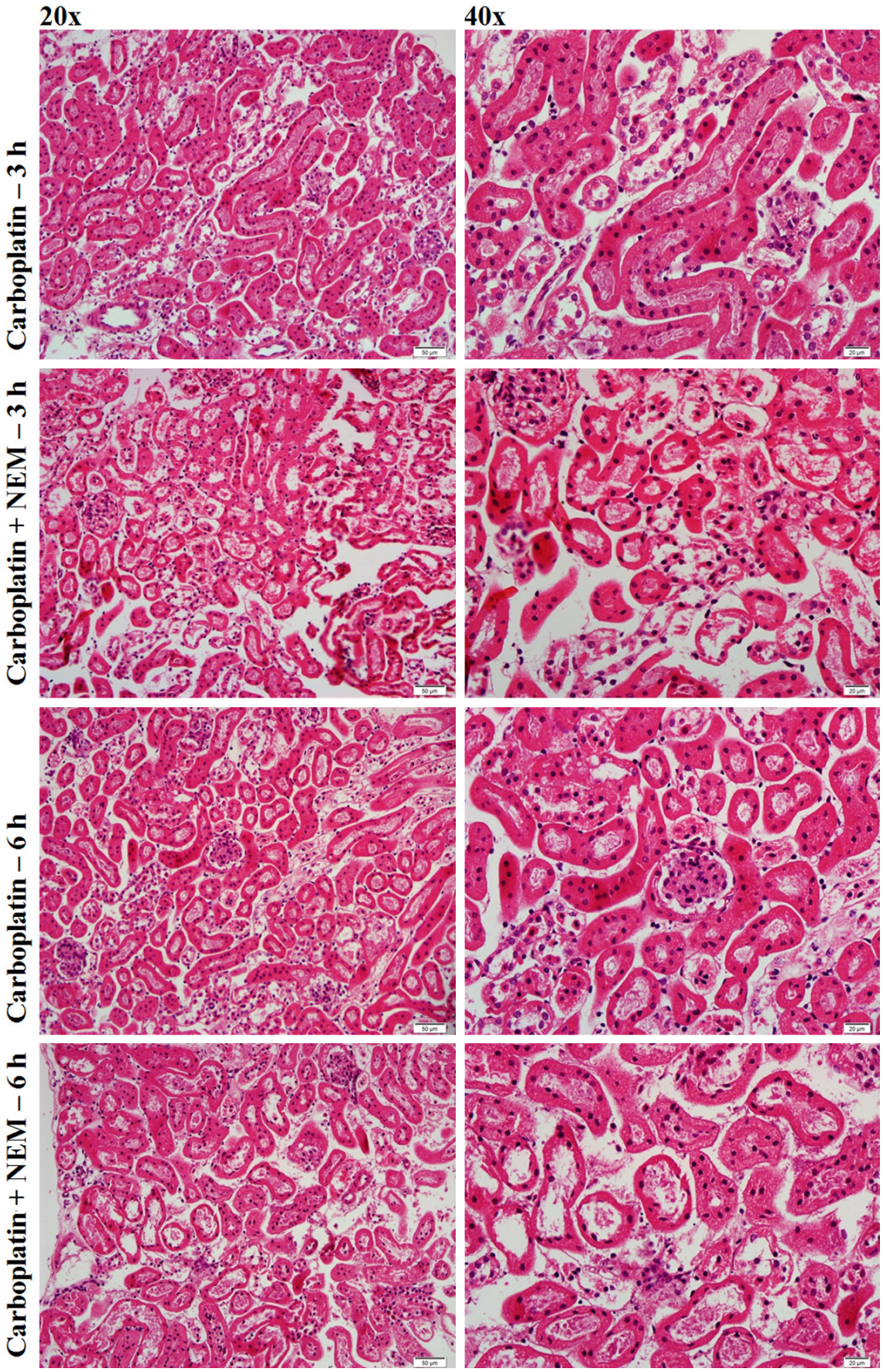

20x
40x
Carboplatin – 3 h
Carboplatin + NEM – 3 h
Carboplatin – 6 h
Carboplatin + NEM – 6 h

Figure 6. Renal histology with carboplatin ± NEM.

phyrin] was used to measure renal cellular respiration following exposure to Pt drug. This method allowed accurate *in vitro* monitoring of renal tissue mitochondrial O_2 consumption over 6 h (**Figures 1**, **2**). The primary objective of the study was to assess renal cellular bioenergetics (the biochemical processes involved in cellular energy metabolism, more specifically cellular respiration and ATP content) following Pt drug therapy. The secondary objective was to explore whether these surrogate biomarkers (cellular respiration and ATP) could be used to assess the nephrotoxicity of various agents. The results show relative stability of renal cellular respiration and ATP in the presence of high-doses of Pt drugs (**Tables 1**, **2**). Thiol depletion (using NEM), on the other hand, resulted in marked Pt-induced nephrotoxicities (**Figures 2**, **3**). These results demonstrate the impact of cellular thiols on the adverse events of Pt therapy. The data also argue for assuring adequate cellular thiols (e.g., nutrition and use of methanethiolate or other cytoprotective agents) prior to administering high-doses of Pt drugs [28-32].

Incubation with NEM was associated with severe ATN (**Figures 3**, **4**). Tubular structures were somewhat similar with cisplatin+NEM *vs.* cisplatin alone (**Figure 5**). Tubular derangements, however, were more prominent with carboplatin + NEM *vs.* carboplatin (**Figure 6**) or oxaliplatin + NEM *vs.* oxaliplatin (**Figure 7**). The correlations between renal histology (structure), respiration (function) and ATP (function) support the use of mitochondrial functions as surrogate biomarkers for studying drug-induced nephrotoxicities.

The mitochondria use energy derived from oxidations in the respiratory chain to generate adenosine 5'-triphoshate (oxidative phosphorylation). These vital organelles also respond to cellular injuries (apoptotic signals) by releasing cytochrome c and other small molecular weight pro-apoptotic molecules from the intermembrane space to the cytosol. In the cytosol, cytochrome c binds to the apoptotic protease activating factor-1 (Apaf-1), triggering the caspase (cysteine-dependent *asp*artate-directed prote*ase*) cascade. Caspase activation leads to mitochondrial perturbation, which involves opening mitochondrial permeability transition and collapsing the electrochemical potential[2, 19]. Thus, induction of apoptosis and mitochondrial dysfunction are highly linked processes.

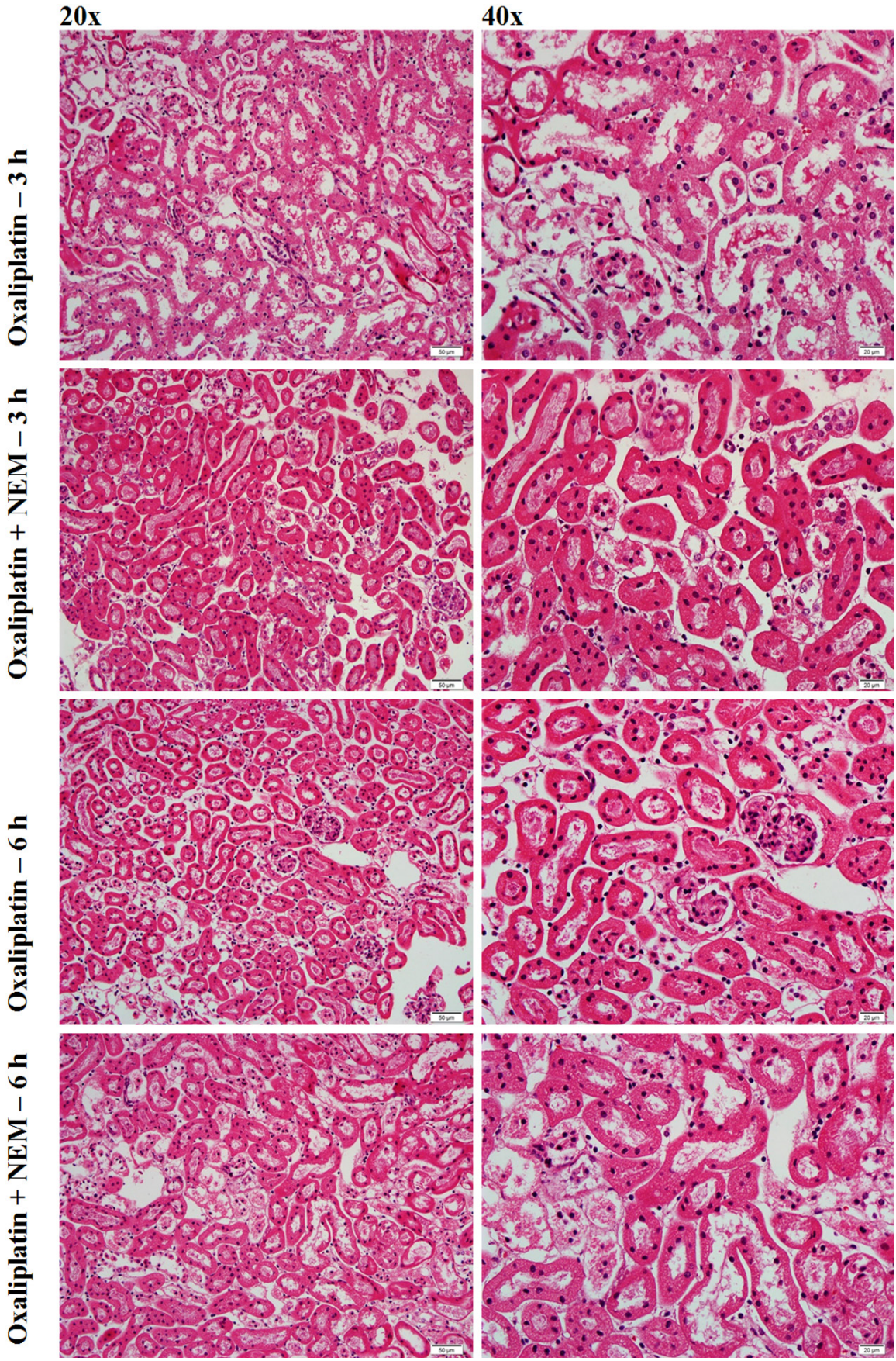
Cellular respiration was relatively preserved in renal tissue treated with Pt drugs (**Figures 1**, **2** and **Tables 1**, **2**). Thus, in the presence of adequate cellular thiols, renal tissue mitochondria are not rapidly targeted by Pt compounds. In Jurkat cells, cisplatin (in contrast to dactinomycin and doxorubicin) inhibited cellular respiration, decreased cellular ATP and induced caspase activity only after 14 h of incubation [19]. The present study investigated *in vitro* incubations for only up to 6 h. The gradual structural and functional *in vitro* deteriorations mandated limiting the incubation time to 6 h (**Figure 3**). Because of this important limitation, the fates of renal cellular respiration and ATP at much longer incubation times remain unknown.

Pt drugs produced rapid deteriorations in cellular bioenergetics (↓respiration/↓ATP) when renal thiols were blocked by NEM (**Tables 1**, **2**). These results point to the importance of renal thiols in detoxifying Pt-based agents. Thus, Pt drugs should be used clinically only if cellular thiols are presumed to be sufficient.

Ligands occupying the Pt coordination spheres influence drug reactivity with target sites. For example, the steric hindrance in carboplatin structure slows its reactivity and cytotoxicity; its six-membered ring (formed between the Pt and the 1, 1-cyclobutanedicarboxylato moiety) adopts a configuration that forces an axial position of the Pt coordination sphere, which delays the reacting nucleophiles from reaching the Pt(II) [13, 27]. The strain in the five-membered ring of oxaliplatin, on the other hand, permits more rapid reactivity [28-30]. Thus, studying Pt compounds requires careful appreciation of the kinetics of their interactions with DNA and with other targeted molecules (including cellular thiols) [31, 32].

Conclusions

Renal tissue is sensitive to thiol depletion, which increases the nephrotoxicity of many agents including platinum compounds. This


20x
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Oxaliplatin – 3 h
Oxaliplatin + NEM – 3 h
Oxaliplatin – 6 h
Oxaliplatin + NEM – 6 h

Figure 7. Renal histology with oxaliplatin ± NEM.

study investigated these aspects of nephrotoxicities, using cellular respiration, cellular ATP and histopathology as surrogate biomarkers. The two most important findings are: (1) Pt nephrotoxicities are significantly increased in the presence of thiol depletion; thus, patients receiving Pt drugs should have adequate cellular thiols. (2) Renal cellular bioenergetics (cellular respiration and ATP) is a useful surrogate biomarker for assessing renal toxicities.

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Disclosure of conflict of interest

None.

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