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doi.org/10.1002/cbdv.202200704

RESEARCH ARTICLE

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The Effects of Sunitinib in Healthy and Cisplatin-Induced Rats

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Sunitinib is a multitargeted kinase inhibitor that inhibits many receptor tyrosine kinases and has been used in the treatment of gastrointestinal stromal tumors, metastatic renal cell carcinoma, and pancreatic neuroendocrine tumors. In this study, the effects of sunitinib given to rats, both alone and after stress with cisplatin, were investigated. The animals were divided into four groups – (1) control group (C) administered interperitoneally with a single dose 0.9% saline, (2) Cis group administered a single dose (7 mg/kg) of cisplatin, (3) Sun group administered 10 mg/kg sunitinib for seven days, and (4) Cis+Sun group administered 10 mg/kg sunitinib for seven days after a single dose (7 mg/kg) of cisplatin. After these applications, the rats were sacrificed, and blood and tissue samples were taken for biochemical and histopathological evaluations. Sunitinib did not show any effect on urea, creatine, and kidney IL1β and TGF-β3 expression levels when administered alone; it increased ALT, AST, and IL-38 levels. When sunitinib was given to the cisplatin-induced rats, it was observed that the increase in ALT, AST, and IL-38 levels increased more than the rats that was given only sunitinib. According to the data obtained, sunitinib does not cause a significant change in kidney tissue under both normal and stress conditions, while it creates stress in liver tissue. In addition, its toxicity in the liver becomes more certain as a result of its combination with cisplatin.

Keywords: cisplatin, IL-38, nephrotoxicity, rat, sunitinib.

Introduction

In recent years, the human genome has been almost entirely sequenced, and in light of this, oncogenes and tumor suppressor genes have intensified, which has led to the emergence of targeted agents that prevent the proliferation and invasion of cancer cells by interfering with specific molecules involved in tumor growth and progression.[1] Sunitib, an oral oxindole, is a multitargeted kinase inhibitor used for this purpose and thus inhibits many receptor tyrosine kinases (RTKs).[2] Tyrosine kinases are inhibited by sunitinib, especially vascular endothelial growth factor receptors 1–3 (VEGFR type 1–3), and others, such as colony-stimulating factor-1 receptor (CSF1R), FMS-like tyrosine kinase-3 (FLT3), glial cell lines-derived neurotrophic factor receptor (RET), platelet-derived growth factor receptors (PDGFR-α and PDGFR-β), and stem cell growth factor receptor (ScGFR or c-KIT).[2,3] The importance of VEGFR1 and VEGFR2 in vasculogenesis and angiogenesis and the presence of PDGFR-β in pericytes that surround the capillary endothelial cells and stabilize the vascular endothelium show how effective sunitib can be.[3] Metastatic renal cell cancers exhibit extensive vascularity. On the other hand, KIT

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RTK has an effect on its own in the oncogenic process of gastrointestinal stromal tumors (GISTs). It is also known that KIT RTK causes cell transformation with has played an important role in the imbalance between increased cell growth and inhibition of apoptosis. These conditions make the cytotoxic effect of sunitib crucial.[2,3]

Cisplatin is an inorganic platinum compound used for chemotherapy purposes in many types of solid cancers, such as bladder, brain, breast, cervical, head and neck, lung, testicular and ovarian cancer, and even melanoma and lymphomas.[4,5] The antitumoral property of cisplatin is that this agent crosslinks with the purine bases in the DNA chain, thus creating a lesion in the DNA as a result of the formation of DNA-platinum adduct, followed by the activation of a series of signal transduction pathways, including ATR, p53, p73, and MAPK. Finally, it is due to the cell's drive to apoptosis.[5,6] The effects of cisplatin in the cell are not limited to this, but it also causes problems such as endoplasmic reticulum stress induction, mitochondrial dysfunction, formation of reactive oxygen species, and inflammation through other chemokines.[7] It is known that cisplatin has many organ toxicities, especially nephrotoxic effects.[7,8] Cisplatin is mostly collected in the kidney and causes tubular cell death and inflammation through the activation of signal pathways.[8]

Cisplatin is frequently used under laboratory conditions to create many different toxicity models, especially nephrotoxicity. Information on how sunitinib works both alone and in the presence of stress is very limited. For this purpose, this study investigated how sunitinib behaves in liver and kidney tissues in rats when administered alone, as well as after induction with cisplatin.

Results

In the comparison between the groups, there was a statistically significant difference in urea levels ($P \leq 0.001$). When compared with the control separately, the urea levels increased in the Cis and Cis + Sun groups. Likewise, the urea level in the Cis and Cis + Sun groups was significantly higher than in the Sun group. The differences between the control and Sun groups and between the Cis and Cis + Sun groups were insignificant (*Table 1*).

The difference was significant in the comparison of creatinine between the groups ($P \leq 0.001$). The creatine levels increased in the Cis and Cis + Sun groups when compared with the control separately. In addition, the urea levels in the Cis and Cis + Sun groups increased compared to the Sun group. The differences between the control and Sun groups and between the Cis and Cis + Sun groups were insignificant (*Table 1*).

The difference in ALT levels between the groups was significant ($P \leq 0.001$). While the difference between the control and Cis groups was insignificant, the ALT levels increased in both the Sun and Cis + Sun groups in the pairwise comparison with these two groups separately. The ALT level in the Cis + Sun group was quite high compared to the Sun group (*Table 1*).

The AST level between the groups was significant ($P \leq 0.001$). There was no difference between the control and Cis groups. In paired comparisons, the AST level measured in the Sun and Cis + Sun groups increased compared to both the control and Cis groups. The AST level in the Cis + Sun group was found to be significantly higher than that in the Sun group (*Table 1*).

The difference in the comparison of IL-38 levels between the groups ($P \leq 0.001$) was found to be significant. While no difference was found between the control and Cis groups, the difference was significant between the Sun and Cis + Sun groups. The difference was significant in the pairwise comparisons between the control and Sun and control and Cis + Sun groups. Similarly, there was a similar significance in the pairwise comparisons between the Cis and Sun and Cis and Cis + Sun groups (*Table 1*).

Table 1. The biochemical values are expressed as the mean values ± standard deviation. In pairwise group comparisons, the base group for comparison is shown in the lower case (for example, a). If the group being compared had a difference of $P \leq 0.001$, one capital letter (for example, A) was used, and if there was a difference of $P \leq 0.05$, two capital letters (for example, AA) were used.

Groups	Urea (mg/dL)	Creatinine (mg/dL)	ALT (u/L)	AST (u/L)	IL-38 (pg/mL)	8-oxodG (ng/mL)
C	40.78 ± 2.05^{a}	0.23 ± 0.02^{a}	26.80 ± 4.02^{a}	66.00 ± 9.77^{a}	$144{,}62 \pm 24{,}45^{a}$	$15{,}36 \pm 2{,}21$
Cis	$133.25 \pm 10.24^{A,B}$	$1.02 \pm 0.20^{AA,BB}$	25.17 ± 3.19^{b}	58.60 ± 2.07^{b}	$149{,}70 \pm 24{,}52^{b}$	$14{,}29 \pm 1{,}37$
Sun	40.14 ± 6.99^{b}	0.20 ± 0.03^{b}	$54.14 \pm 8.47^{A,B,c}$	$86.08 \pm 12.68^{AA,B,c}$	$396{,}39 \pm 78{,}96^{A,B,d}$	$13{,}44 \pm 1{,}98$
Cis + Sun	$135.33 \pm 17.90^{A,B}$	$0.91 \pm 0.37^{AA,BB}$	$130.20 \pm 26.62^{AA,BB,CC}$	$243.40 \pm 63.75^{AA,BB,CC}$	$656{,}04 \pm 149{,}88^{A,B,DD}$	$14{,}39 \pm 1{,}85$

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In the comparison between groups, no difference was observed in terms of 8-oxodG; therefore, no pairwise comparison could be made (*Table 1*).

The difference between the groups in the IL-1β comparison was significant ($P \leq 0.001$). In paired comparisons with the control, the expression level was increased in the Cis and Cis + Sun groups (3.38 ± 0.94 and 3.07 ± 0.60 fold, respectively). The expression levels in the Cis and Cis + Sun groups were also higher than the Sun group (1.01 ± 0.23 fold) (*Figure 1*).

The difference between the groups in TGF-β3 comparison was significant ($P \leq 0.001$). Compared with the control, no significant change was observed in the Sun group (1.28 ± 0.16 fold). However, TGF-β3 expression levels in both Cis and Cis + Sun groups were significantly increased in the pairwise comparison with the control and Sun groups (4.28 ± 0.52 and 3.90 ± 0.73 fold, respectively) (*Figure 1*).

The liver tissues of the rats in the control group had a normal histological appearance. While mild hemorrhagic areas and degenerative hepatocytes were seen in the Cis group, moderate hemorrhagic areas and degenerative hepatocytes were observed in the Sun and Cis + Sun groups (*Figure 2*). The kidney tissues of the rats in the control group had a normal histological appearance. Mild intertubular hemorrhage areas were seen in the Sun group. In the Cis and Cis + Sun groups, severe intertubular hemorrhage and severe tubular degeneration were observed (*Figure 3*).

Discussion

This study looked at the effects of sunitinib alone and in the presence of cisplatin-induced stress. For this purpose, serum biochemical markers and expression parameters in kidneys were used. During the literature review, it was found that cisplatin causes toxicity in many different cell and tissue types, and this feature is the biggest problem in chemotherapy. One of the tissues where cisplatin accumulates most besides the liver and prostate is the kidney, and its most negative effect is nephrotoxicity.[7–9]

The cisplatin dose to be used to induce nephrotoxicity was determined to be 7 mg/kg.[10,11] It has been reported that sunitinib causes mortality and moribundity in rats at a dose of 15 mg/kg/day.[12] In another study, it was noted that sunitinib exhibited toxic effects in rats at a dose of 10 mg/kg/day.[13] In line with these results, sunitinib was administered to rats at a dose of 10 mg/kg/day in our study.

It has been reported that IL-38 is a cytokine that has pro-inflammatory and anti-inflammatory effects and is expressed in different tissues, such as the heart, placenta, fetal liver, spleen, thymus, and tonsil, and is generally associated with rheumatic diseases.[14,15] Information about IL-38 in the literature is extremely limited; its role in a stress situation is unknown. However, in a study conducted in mice with liver damage, it was shown that the levels of IL-38 along with ALT and AST increased parallelly with the stress state.[16,17]

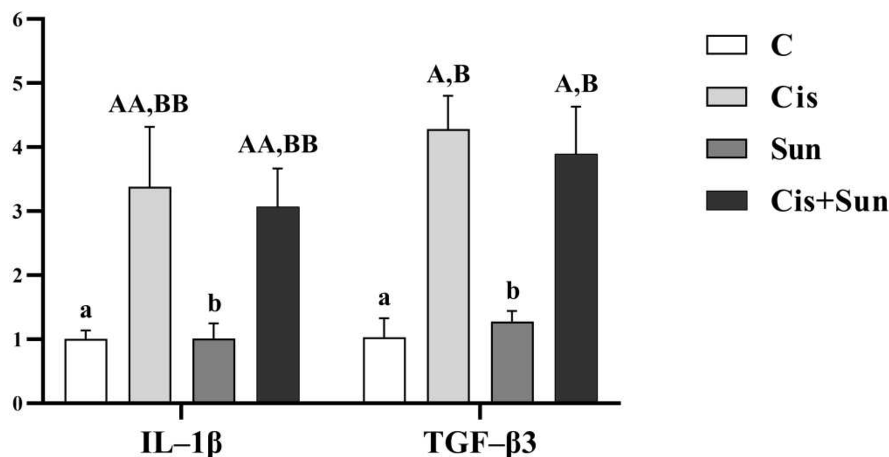

Figure 1. Relative gene expression of IL-1b and TGF-β3 expressed as the mean values ± standard deviation. In pairwise group comparisons, the base group for comparison is shown in the lower case (for example, a). If the group being compared had a difference of $P \leq 0.001$, one capital letter (for example, A) was used, and if there was a difference of $P \leq 0.05$, two capital letters (for example, AA) were used.

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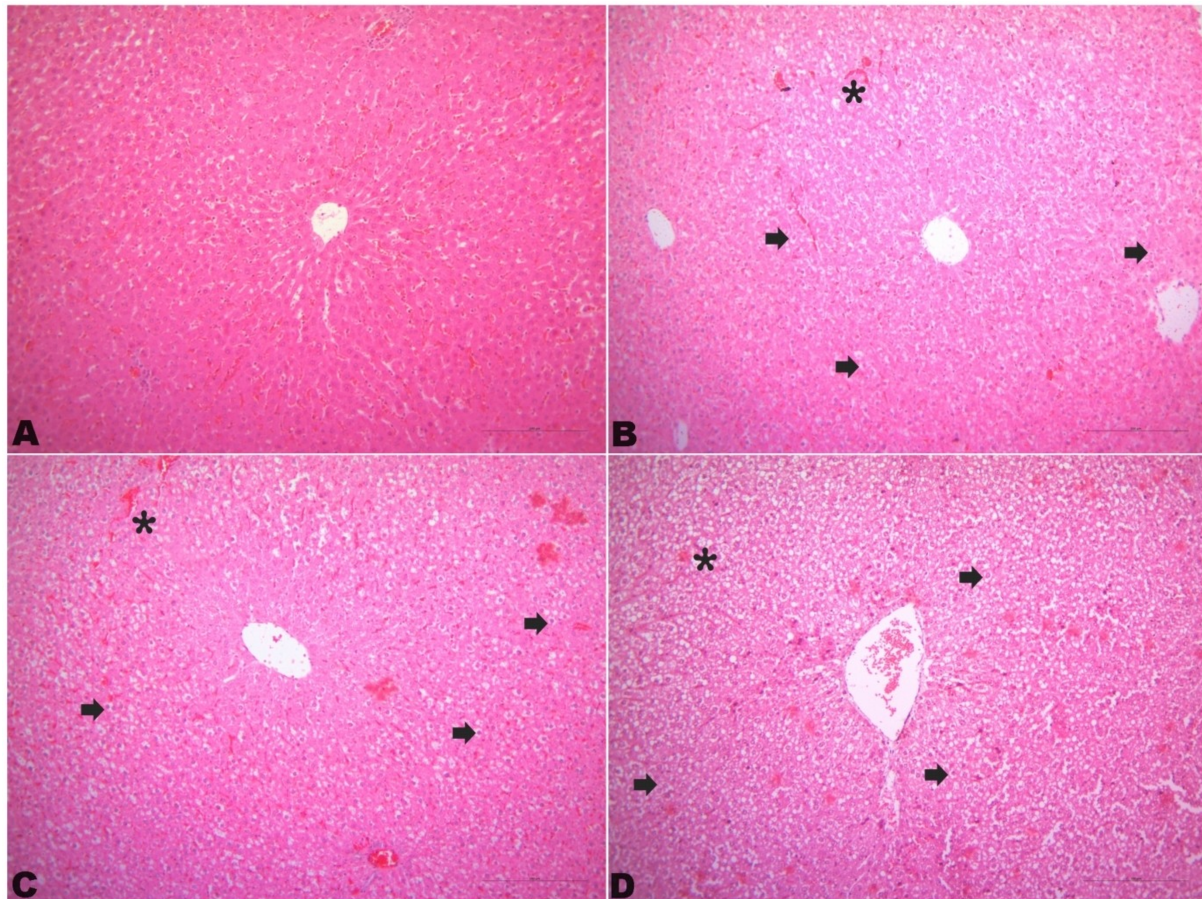

Figure 2. Histopathological findings of liver tissues. A. Control group, normal histological appearance B. Cis group, mildly hemorrhagic areas (*) and degenerative hepatocytes (arrowhead). C. Sun group, moderately hemorrhagic areas (*) and degenerative hepatocytes (arrowhead). D. Cis + Sun group, moderately hemorrhagic areas (*) and degenerative hepatocytes (arrowhead). H&E

While an increase in serum ALT and AST is considered a significant predictor of liver dysfunction due to the deterioration of liver cell membrane permeability, an increase in urea and creatinine levels indicates the presence of renal dysfunction. Experimentally, Palipoch and Punsawad[18] reported that the hepatotoxic and nephrotoxic effects of cisplatin vary depending on the dose and time after administration. These researchers showed that serum ALT and AST levels decreased with time, while BUN and creatinine increased in rats that were given cisplatin. Considering the dynamic structure of the liver, the accumulation of cisplatin in the kidney, and the kidney being the excretory organ of this agent, these results can be considered normal. Similar to the results of this study, according to our data, the normal levels of ALT, AST, and IL-38 in the serum obtained as a result of sacrificing the rats given only cisplatin 7 days later resulted in a general improvement in the organism depending on time. However, urea and creatinine levels were higher in the Cis group than in the control group, indicating that nephrotoxicity is still present in this group.[19,20]

When sunitinib alone was applied, it was observed that the urea and creatinine levels did not change compared to the control group. Similarly, when sunitinib was given to rats induced by cisplatin, the urea and creatinine levels did not change compared to the cisplatin-only group. This shows that sunitinib does not affect urea and creatinine levels either under normal conditions or under stress conditions. In addition, it was noted that sunitinib increased ALT, AST, and IL-38 both when administered alone and when the rats were induced with cisplatin. What is more remarkable is that ALT, AST, and IL-38 levels were higher in the rats induced with cisplatin than in the rats treated with sunitinib alone. These data show that cisplatin and sunitinib may have shown a

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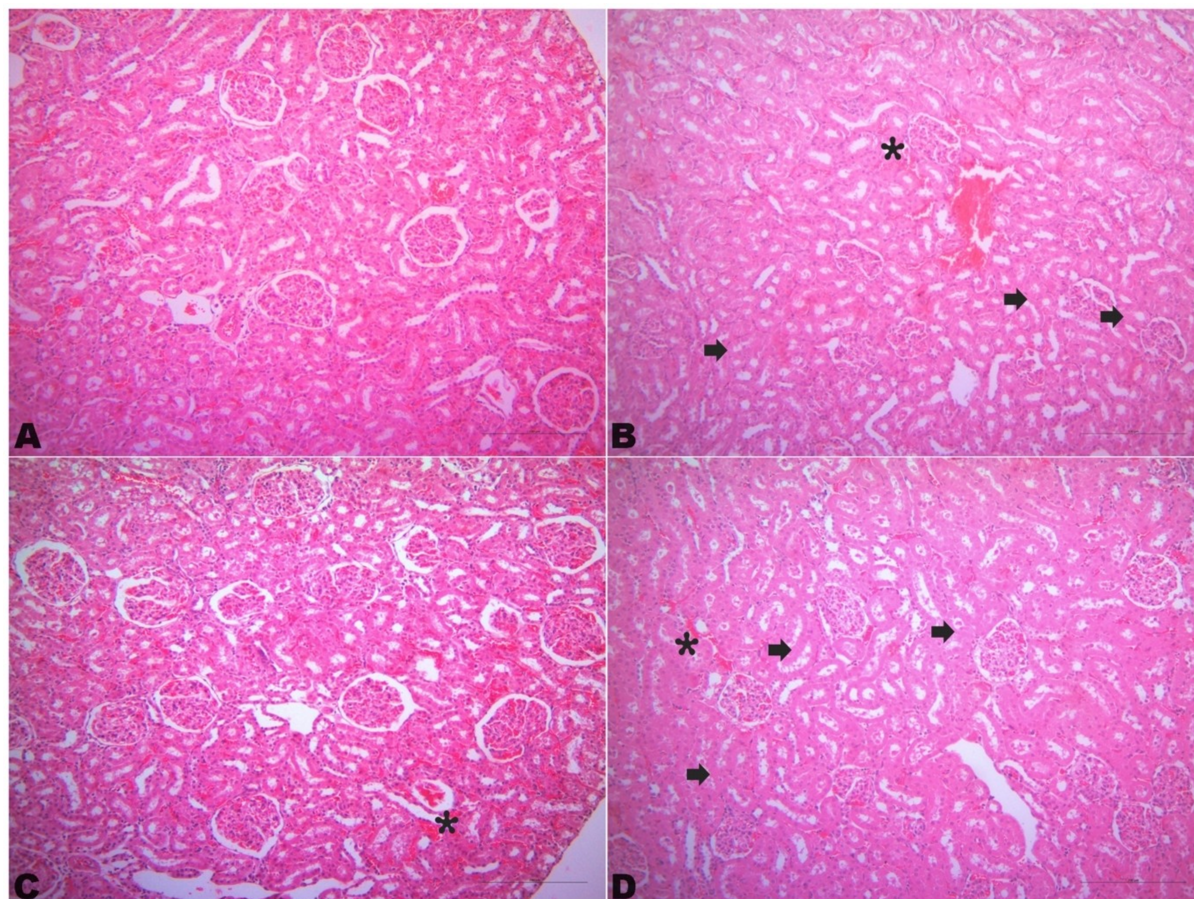

Figure 3. Histopathological findings of kidney tissues. A. Control group, normal histological appearance. B. Cis group, severe hemorrhage in intertubular areas (*) and degenerative tubules (arrowhead). C. Sun group, mildly hemorrhage in intertubular areas (*). D. Cis + Sun group, severe hemorrhage in intertubular areas (*) and degenerative tubules (arrowhead). H&E

synergistic effect in terms of the formation of liver toxicity, or it can be assumed that sunitinib adversely affects the healing process in the liver, considering that the stress created by cisplatin in the organism is relieved over time. In previous studies, similar to the data we obtained, it was shown that sunitinib did not change BUN and creatinine levels but rather increased ALT and AST.[21,22] Suddek[21] also reported that BUN and creatinine levels increased with cisplatin and decreased when sunitinib was given, but our data show that sunitinib has no effect on the urea and creatinine caused by cisplatin. In this respect, our study is the first to show that sunitinib does not affect urea and creatinine levels in either healthy or stressed rats. The level of 8-oxodG, which is a marker of oxidative DNA damage, was also investigated, and the differences in all groups were statistically insignificant when compared to the control.

Similar to the data we obtained, it was previously shown that the level of IL-1b, a marker of inflammation, increased in the kidney tissue of rats induced with cisplatin.[20] Information on the effect of sunitinib on kidney tissue damage is very limited. According to Al-Harbi et al.,[23] sunitinib caused damage to the renal tissue at a dose of 50 mg/kg in rats, while in another study,[24] when sunitinib was administered at a dose of 100 mg/kg, it reduced NF-κB and TNF-α mRNA levels. Korashy et al.[24] reported that sunitinib shows antioxidant and anti-inflammatory properties by increasing CAT and SOD mRNA levels. According to the data we obtained, sunitinib does not show any effect on the IL-1b level when given alone. Although the expression level decreased when sunitinib was administered to the rats that were given cisplatin, there was no statistically significant difference compared to the group that was only administered cisplatin. The fact that the data we obtained on the basis of kidney tissue

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is different from previous studies may be because the dose we applied (10 mg/kg) and the duration of application were not the same as in these studies.

Fibrosis occurs because of the progression of chronic injuries to the kidney caused by cisplatin and other toxic agents.[19] TGF-β has a notable role in promoting conduction fibrosis.[25] Although increased TGF-β1 expression level indicates fibrosis, TGF-β2 and TGF-β3 expression levels were also raised in fibrosis formation.[26–29] In the literature, unlike other TGF isoforms (TGF-β1 and TGF-β2), information about TGF-β3 is very limited and controversial. A recent study reported that TGF-β3 may have antifibrotic and renoprotective properties; thus, it maintains renal homeostasis by inhibiting TGFβ1 activity.[30] However, Yu et al.[31] reported that all TGF isoforms, including TGF-β3, have fibrogenic effects in renal cells, and the blockade of these isoforms will have a good therapeutic effect in reducing renal fibrosis. Another study noted that the expression level of TGF-β3 decreased in parallel with the improvement in kidney fibrosis.[25] From the perspective of tissue toxicity, TGF-β3 expression heightened due to the testicular damage induced by $CdCl_2$ in mice.[32] According to the data obtained in our study, TGF-β3 expression followed a parallel course with IL-1b. The raised expression of TGF-β3 in the Cis group compared with the control may indicate that fibrosis occurred after cisplatin administration. In addition, the absence of a higher TGF-β3 level in the Sun group compared to the control group and the fact that the TGF-β3 expression level in the Cis + Sun group did not statistically differ from the Cis group reveal that sunitinib did not change the expression level of TGF-β3.

Conclusion

In this study, it was noted that when sunitinib was administered alone, it caused toxicity in the liver, and when it was given to rats treated with cisplatin, the toxicity increased. This may be because sunitinib has a synergistic effect with cisplatin, or its administration prevents cisplatin-induced liver damage from healing over time. It was also noted that sunitinib did not cause any changes in kidney tissue when administered alone or when administered to rats induced by cisplatin. These obtained data are in agreement with our histopathological findings. Considering that the information about sunitinib is still insufficient, we think that our data will contribute to the literature.

Experimental Section

Chemicals

Cisplatin (Cisplatin-Ebewe®, 100 mg/100 mL) was purchased from Liba Lab (Istanbul, Türkiye), sunitinib (Sutent, 50 mg) and ketamine (Ketalar, 500 mg/10 ml) were purchased from Pfizer Drug Co. (Istanbul, Türkiye), and xylazine (XylazinBio 2%, 20 mg/m ml) was purchased from Bioveta (Ankara, Türkiye).

Animals

This study was carried out with the approval of the Local Ethics Committee of Animal Experiments, Atatürk University, and all the processes throughout the study were carried out by adhering to the animal care and application rules of the Türkiye National Animal Experiments Ethics Committee (date of approval: September 28, 2017; approval number: 3126). Twenty-eight male albino Wistar rats (*Rattus norvegicus*) weighing 200–220 g were obtained from the Experimental Application and Research Center, Atatürk University Faculty of Medicine. The animals were kept in 'plastic breeding cages' both in their normal routine lives and during the experiment and were given ad libitum access to water and basal food to establish standard chow. Also, a 12-h light/dark cycle, 21 ± 1 °C temperature, and a relative humidity of approximately 60% were provided in living environments to create controlled experimental conditions. The rats were randomly divided into 4 groups, 7 in each group. The experimental protocol consisted of the following groups:

Control (C)
Cis group: 7 mg/kg cisplatin; Sun group: 10 mg/kg sunitinib; Cis + Sun group: 7 mg/kg cisplatin + 10 mg/kg sunitinib

A single dose 0.9% saline was injected intraperitoneally to the rats in the C group. Cisplatin was dissolved in 0.9% saline and administered intraperitoneally as a single dose to the animals in the Cis and Cis + Sun groups. In the Sun group, sunitinib was administered orally for 7 days. In the Cis + Sun group, sunitinib was administered orally for 7 days, with the first dose given 1 h after cisplatin administration. At the end of these applications, an anesthesia cocktail consisting of ketamine (60 mg/kg) and xylazine (7.5 mg/kg) dissolved in 0.9% saline was administered intraperitoneally to all animals and then euthanized by exsanguination. Kidneys taken from each sacrificed rat and washed with 0.9% saline were digested with a

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homogenizer (IKA Ultra-Turrax T25 basic homogenizer, Germany). To obtain serum, blood samples (~5 ml) were centrifuged at 2500 rpm for 15 min (Eppendorf 5430R, Hamburg, Germany). For biochemistry and PCR analysis, homogenizations and serum samples were kept at −80 degrees.

Biochemical Analysis

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatine, interleukin 38 (IL-38), and 8-Oxo-2′-deoxyguanosine (8-oxodG) measurements were made using serum samples taken from the rats. The kinetic urease/glutamate dehydrogenase method was used to determine the urea level, and the uncompensated Jaffe method was used to determine the creatine level. ALT and AST were measured according to IFCC with pyridoxal phosphate activation. These measurements were performed with an Olympus AU640 autoanalyzer (Olympus, Kobe, Japan). The quantification of IL-38 and 8-oxodG was done with a Multiskan Sky Microplate Spectrophotometer (Thermo Scientific, Waltham, MA, USA) using the Sandwich ELISA kit. The kits were procured from Sinogeneclon Biotech Co. Ltd. (Hangzhou, China), and the procedures for these kits were followed during the study (Catalog numbers SG-21170 and 21168, respectively).

Quantitative Real-Time PCR

GeneAll® Hybrid-RTM (Cat No: 305-101, South Korea) total RNA purification kit was used for total RNA isolation from kidney homogenizations. Complementary DNA (cDNA) synthesis from the obtained RNA was performed with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California 94404, USA). Both total RNA purification and cDNA synthesis were performed under the kit procedure. Quantification of the cDNA concentration was conducted with μDrop™ Plate integrated into a Multiskan Sky Microplate Spectrophotometer (Thermo Scientific, Waltham, MA USA). In addition, the reverse transcription and quantitative real-time PCR (qRT-PCR) processes were conducted using the QuantStudio 5 Real-Time PCR instrument (Thermo Fisher Scientific, Waltham, MA, USA), and SYBR Green-based expression analysis in QuantiTect Primer Assays (Qiagen, Hilden, Germany) was used during qRT-PCR. The total volume for all samples was determined as 20 μl. This volume consisted of 5 μL of cDNA, 12.5 μL of the master mix, and 2.5 μL of the primer solution. The catalog and NCBI reference sequence numbers of the primers used were as follows:

ACTB1 (Rn_Actb_1_SG):
QT00193473 and NM_031144.3

IL1β (Rn_Il1b_1_SG)::
QT00181657 and NM_031512.2

TGFβ3 (Rn_Tgfb3_1_SG)::
QT00177065 and NM_013174.2

The ACTB1 gene was used for normalization. The PCR cycling conditions applied to determine gene expression were temperatures of 95 °C for 15 min, 94 °C for 15 seconds (40 cycles), 55 °C for 30 seconds (40 cycles), and 72 °C for 30 seconds (40 cycles). In addition, the melting curve analysis was performed to understand whether the generation of amplification products took place immediately after this process. The cycling conditions of the melting curve analysis were 95 °C for 15 seconds, 60 °C for 1 min, and 95 °C for 15 seconds. After the qRT-PCR process was performed, the data were evaluated using the $2^{-\Delta\Delta CT}$ method.

Histopathological Evaluation

Kidney and liver tissues taken from the rats were fixed in 10% neutral-buffered formaldehyde solution for 2 days. Afterwards, the tissues were embedded in paraffin blocks after standard alcohol-xylol treatments. Paraffin-blocked tissues were divided into 5-mm-thick sections using a microtome (Leica RM2145; Leica Instruments, Nussloch, Germany). For each tissue sample, 10 randomly selected sections were deparaffinized and stained with hematoxylin-eosin. The histopathological evaluation was conducted by a pathologist who was blinded to the study groups through a light microscope (Zeiss Primo Star with an integrated Carl Zeiss Axiocam ERc5s; Carl Zeiss AG, Oberkochen, Germany).

Statistical Analysis

Statistical analysis of the data was performed using IBM SPSS 22 (Armonk, NY: IBM Corp.). Descriptive statistics were given as mean ± standard deviations. The conformity of the variables to the normal distribution was checked using the Kolmogorov-Smirnov test of normality. Comparisons between the groups were made with a one-way ANOVA. Homoge-

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neity of variance was evaluated with Levene's test. As a post hoc test, Tukey HSD was used in cases where variance homogeneity was provided, and the Games-Howell test was used in cases where it was not. The value of $p < 0.05$ was accepted as statistically significant.

Acknowledgements

This investigation was partially supported by a Research Fund of Erzincan Binali Yıldırım University (SAG-A-070114-0051).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Author Contribution Statement

Research concept and design: LD, MG; Data collection: LD, MG, HT, EMA, OK; Statistical analysis: YKA; Writing: MG; Review and editing: LD, MG, HT, EMA; Funds collection: LD; All authors have read and agreed to the published version of the manuscript.

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Received August 2, 2022
Accepted January 4, 2023

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