Exp Biomed Res 2022; 5(1): 10-22
Doi: 10.30714/j-ebr.2022173847

Experimental Biomedical Research **Original article**

Riluzole and ranolazine application of prostate cancer: Cancer related testicular and liver tissue damage

Pinar Koroglu Aydin[1], Ilknur Bugan[2]

[1]*Department of Histology and Embryology, Haliç University, Faculty of Medicine, Istanbul, Turkey*
[2]*Department of Biology, Istanbul University, Faculty of Science, Vezneciler, Istanbul, Turkey*

ABSTRACT

Aim: In this study, utilizing the in vivo Copenhagen rat model possessing prostate cancer, we studied the possible impact of tumorigenesis on testes and liver morphology and whether riluzole (RIL) and ranolazine (RNL) treatment would have any affect or not.

Method: Male Copenhagen rats were divided into four groups: 1) Control group, 2) Cancer group, 3) Cancer + 10 µM Riluzole 4), and Cancer + 2.5 µM / 5 µM Ranolazine group. The tissue samples of testes and liver were taken and processed for light microscopy, including staining with hematoxylin and eosin.

Results: In the cancer group, degenerated seminiferous tubules, cell remnants in the lumen were shown in the testis, and a decrease in the spermatogenic cell line was found. The deterioration in these parameters was milder in the treatment groups and an increase in the number of normal tubules was found. In the cancer group, pyknotic nucleus, mononuclear cell infiltration, hyperemia, vacuolization, disrupted arrangement of hepatocyte plates, sinusoidal dilatations, and degenerated hepatocytes were observed in the liver. However, there was a slight damage in cancer + 10 µM RIL, cancer + 2.5 µM RNL, and cancer + 5 µM RNL groups. Properly hepatocyte arrangement and sinusoidal enlargement were observed.

Conclusions: This treatment can be considered a promising protective adjuvant candidate for testes and liver tissue in prostate cancer or cancer therapy-related damage.

Key words: Dunning prostate cancer model, testes, liver, riluzole, ranolazine, mat- lylu cells.

✉ *Pinar Koroglu Aydin*
Department of Histology and Embryology,
Haliç University, Faculty of Medicine, Istanbul, Turkey
E- mail: pinarkoroglu@halic.edu.tr
Received: 2021-06-22 / Revisions: 2021-08-17
Accepted: 2021-09-21 / Published online: 2022-01-01

Introduction

The drug repurposing consist of the investigation of off-target effects of existing drugs for new therapeutic purposes. Some drugs are currently in clinical use and may be renamed off-label as anticancer agents or in cancer-related several problems.

Riluzole (RIL) is a Food Drug Administration (FDA) approved drug for the treatment of amyotrophic lateral sclerosis. RIL also has neuroprotective, anticonvulsant, analgesic, anesthetic, anti-ischemic, and sedative properties. They searched the effects of cell growth and tumorigenesis to repurpose it for the treatment of cancer. RIL is accepted to act by indirectly inhibiting glutamate signaling. However, the specific effects of RIL in breast cancer cells are not well understood [1]. It has been found out that RIL acts as an anti-invasive on rat prostate cancer cells [2].

Cancer cells use a variety of growth signaling pathways to acquire an advantage over normal cells in terms of proliferation. Many types of cancer also express glutamate receptors, suggesting that glutamate may play an important mission. Glutamate signaling is used by breast, prostate, and skin cancers to increase their growth. Because glutamate signaling is so important for tumor growth, it is thought that it will be crucial to investigate the mechanisms underlying glutamate signaling and discover strategies to interrupt this signaling to prevent tumor growth. Glutamate secretion was first shown to be inhibited in brain tissue by a drug, RIL. It has been shown to block sodium channels and glutamate signaling. Breast cancer cells that are triple negative are treated RIL was found to inhibit cell proliferation. In addition, for brain, skin, breast, and prostate cancers, it has been shown to inhibit cancer cell proliferation in culture or in xenograft models. In a melanoma clinical trial, riluzole reduced tumor size in certain patients. RIL also demonstrated some clinical advantages in a phase II trial person with melanoma [3].

Ranolazine (RNL) is an FDA-approved anti-anginal drug with an anti-ischemic activity that has also been shown to have anti-arrhythmic properties due to prevention of the late sodium current in cardiomyocytes [4,5]. It has been suggested that RNL has been shown to be an antagonist against any experimental heart function failure, including doxorubicin cardiotoxicity, by decreasing the reactive oxygen species content. In studies conducted with the prostate and breast cancer in vivo models, it has been revealed that RNL can have an anti-metastatic effect [6,7].

RIL is predominantly metabolized by the hepatic microsomal cytochrome P450 in extrahepatic tissues [8]. In parallel, RNL is metabolized by the cytochrome P-450 system in the liver [9]. Regarding this issue, many factors such as various mediator molecules that play a role in the immune response during the development process of cancer, chemotherapeutic drugs used in cancer therapy, and ionizing radiation used in radiotherapy can cause damage to organs such as the liver and testes in our study [10-12].

In a study examining the pharmacological blockade of lipid oxidation with RNL in prostate cancer models, oral administration of RNL (100 mg/kg for 21 days) was shown to result in decreased tumor CD8+ T-cells, increased macrophages, and decreased blood myeloid immunosuppressive monocytes [13].

The Dunning rat prostate cancer model has now become a useful model that is utilized in the research of androgen-independent prostate cancer and metastasis biology in in vitro and in vivo [14]. In this study, the Dunning model was formed in Copenhagen rats using strongly metastatic Mat-Lylu cells via subcutaneous injection [15].

In our study, we aimed (1) to show the liver and testicular damage that we predicted to occur in the prostate cancer model, (2) to evaluate how effect of our drugs on these tissues, occur damage by histopathological assessments.

No study in the literature addresses testicular or liver damage in experimental or clinical models with cancer or prostate cancer. In our study, it is predicted that RIL and RNL, which continue to be used for non-cancer-related different treatments, will provide advantages in terms of cost and time with the repositioning strategy as a drug in the damage caused by cancer.

Materials and methods

Cell culture

Mat-LyLu cells were grown in RPMI culture medium (RPMI-1640, Gibco; Life Technologies, USA), supplemented with 1%

fetal bovine serum (FBS) (Gibco), 2 mM L-glutamine (Gibco; Life Technologies, USA), and 250 nM dexamethasone (Sigma; Sigma-Aldrich, USA) Cells were cultured at 37ºC in the humidified 5% CO_2 incubator [16].

Animals and prostate cancer model

Male Copenhagen rats (provided from Tubitak Marmara Research Center, Kocaeli, Turkey) were used. The animals were kept in 12:12-hr day-night cycle at 22-25ºC and fed with pellet food and water. To induce tumors (in cancer group and cancer + RIL or RNL groups), $2x10^5$ Mat-LyLu cells were inoculated subcutaneously into the right flank of each rat with isoflurane (Abbott; Queenborough, UK).

After the body weights were recorded, Mat-LyLu injection was inoculated subcutaneously into the upper right front extremity of each rat in the experimental groups. The volume of cell suspension to be injected into the rats was adjusted to be in the range of 0.10 – 0.50 ml. All operations performed on animals (from maintenance to dissection) were carried out under the ethical regulations and specific permission of Istanbul University (I.U. HADYEK, number: 2010/116).

Administration of drugs and experimental design

In the project study, the use of at least 6 subjects for the control group, cancer and treatment groups was calculated by performing power analysis with the pass 2008 program, in order to create a Dunning cancer model at α=0.05 significance level, to obtain 0.8% power.

Male Copenhagen rats were divided into four experimental sets. There are 6 rats in each all group. 1) Control group: Physiological saline (0.9% NaCl) was applied to rats with gavage (1 ml solutions) every other day without tumor inoculation, 2) Cancer group: After the inoculation of Mat-LyLu cells, 0.9% NaCl was applied to rats orally, 3) Cancer+RIL group: 10 µM RIL (in an equal volume of physiological saline) was administrated with gavage every other day for 22 days, following inoculation of Mat-LyLu cells, 4) Cancer+RNL group: Respectively, 2.5 µM and 5 µM RNL doses was applied in the same way and duration.

Monitoring of primary tumors and dissection

The primary tumorigenesis was monitored daily. The dimension of the primary tumor was measured with calipers every day. “Tumor volume” was evaluated as (shortest diameter) 2 × (longest diameter) 1/2 [17].At the 22nd day after the cell inoculation, the primary tumor, testes and liver were dissected.

Histopathological evaluations

The testes and liver tissues were immersed in Bouin's fixative and processed routinely. The hematoxylin and eosin (H&E) tissues were analyzed. At a magnification of x200, five identical locations were chosen and analyzed. Normal, regressive, degenerative, or atrophic tubules were classified as normal, regressive, degenerative, or atrophic tubules based on Hess' data [18]. Normal tubules have normal spermatogenesis and blood testes barrier morphology. Seminiferous tubules with one or more defects make up regressive tubules. Degenerative tubules indicates irregular arrangements and atrophic tubules perform only sertoli cells.

The semi-quantitative histological assessment of liver damage was done using modified histological criteria described earlier [19]. Five similar areas were chosen and investigated at ×400 magnification. The hepatic injury, based on the disrupted arrangement of hepatocyte plates, sinusoidal dilatation, hyperemia, vasocongestion, mononuclear cell infiltration,

pyknotic nucleus, degenerated hepatocytes were scored by using a scale ranging from 0 to 3 (0: none; 1: mild; 2: moderate; and 3: severe) for each criterion. The score was done for all groups. They were photographed by an Olympus CX41 microscope fitted with an Olympus DP71 digital camera DP71, Tokyo, Japan). The images were observed under a light microscopy on the 10x magnification.

Statistical analysis

Graph-Pad Prism 3.0 (GraphPad Software, San Diego, CA, USA) program was used and the data were analyzed by using one-way analysis of variance (ANOVA). The difference between the groups were determined with Tukey's multiple comparison tests.

Results

Primary tumorigenesis and monitoring of primary tumors

The primary tumors were first palpable on the 12th day (in cancer group) and the 16th day (RIL treatment group) d their volumes increased. There was an insignificant decrease in the tumor weights within the cancer + RIL group compared to the cancer group. RNL administration was decreased insignificantly in the tumor weights compared to the cancer group (Figure 1).

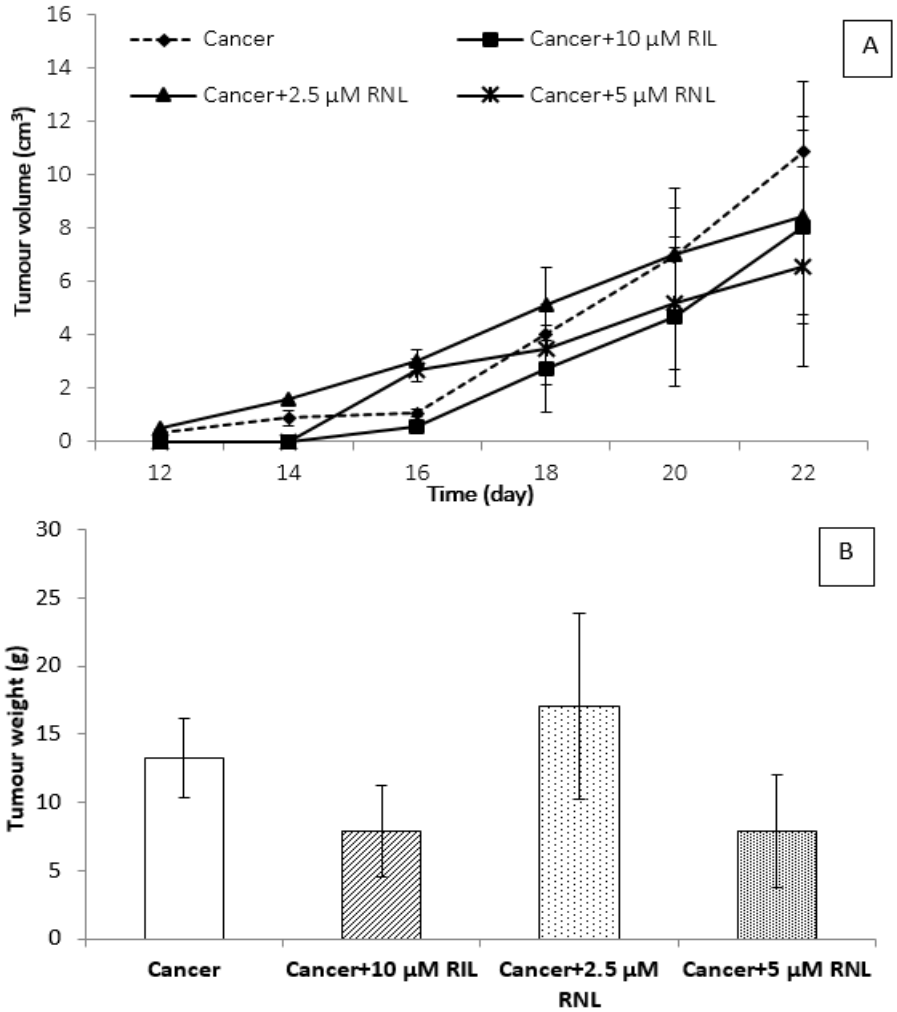

Figure 1. The development of primary tumor after inoculation of Mat-LyLu cells, **A.** Time courses of tumor volume and effects of RIL and RNL (2.5 µM and 5 µM) compared with cancer group. **B.** Tumor weights at the end of the experiment in groups.

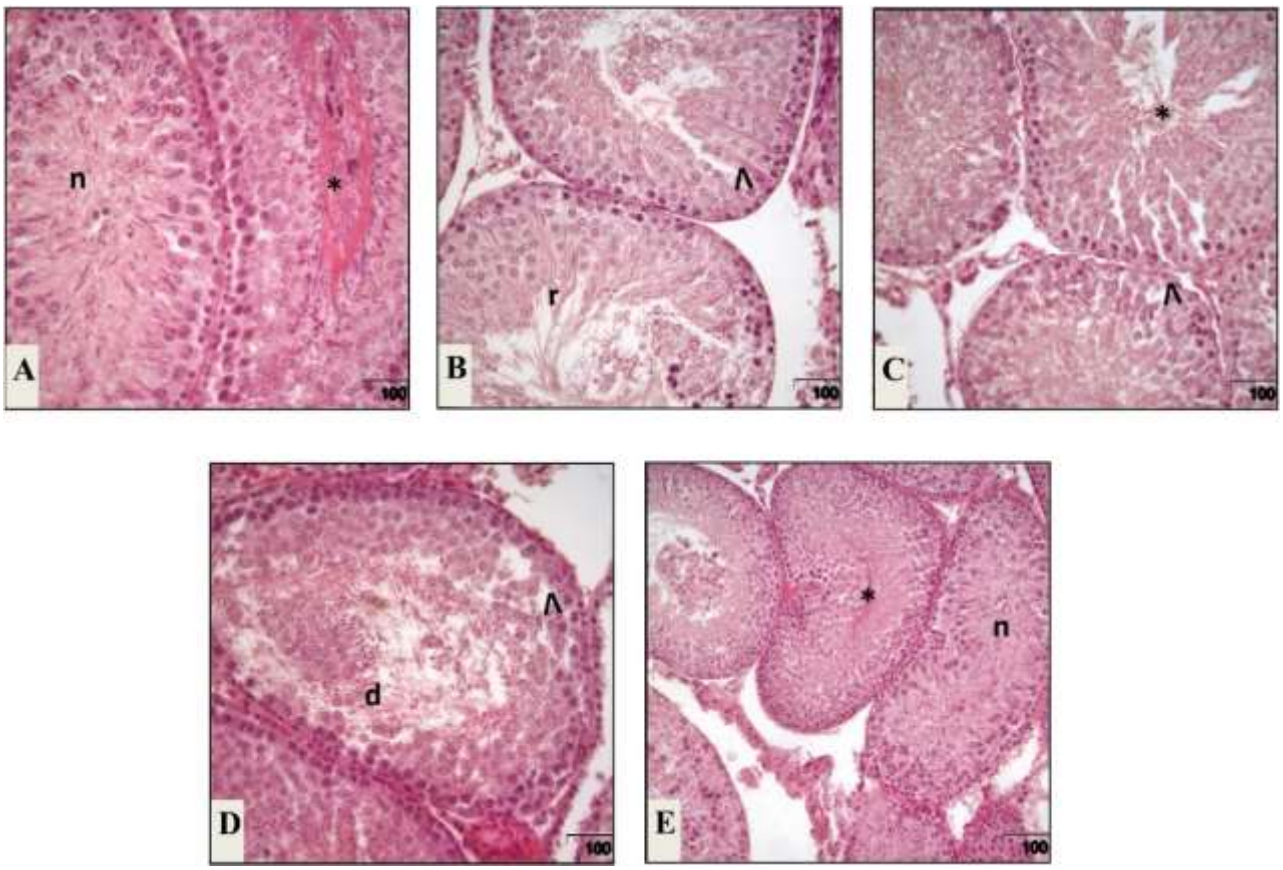

Figure 2. Testes tissue histological appearance: control group **(A)**, cancer group **(B)**, cancer + 10 µM RIL group **(C)**, cancer + 2.5 µM RNL group **(D)**, cancer + 5 µM RNL group. **(E).** Normal morphology in seminiferous tubules (n), spermatogenic cells (^), spermatozoa (*) in lumen of the seminiferous tubules, degenerative (d) and regressive (r) seminiferous tubules, H&E staining, Bar: 100 µm, x10 magnification.

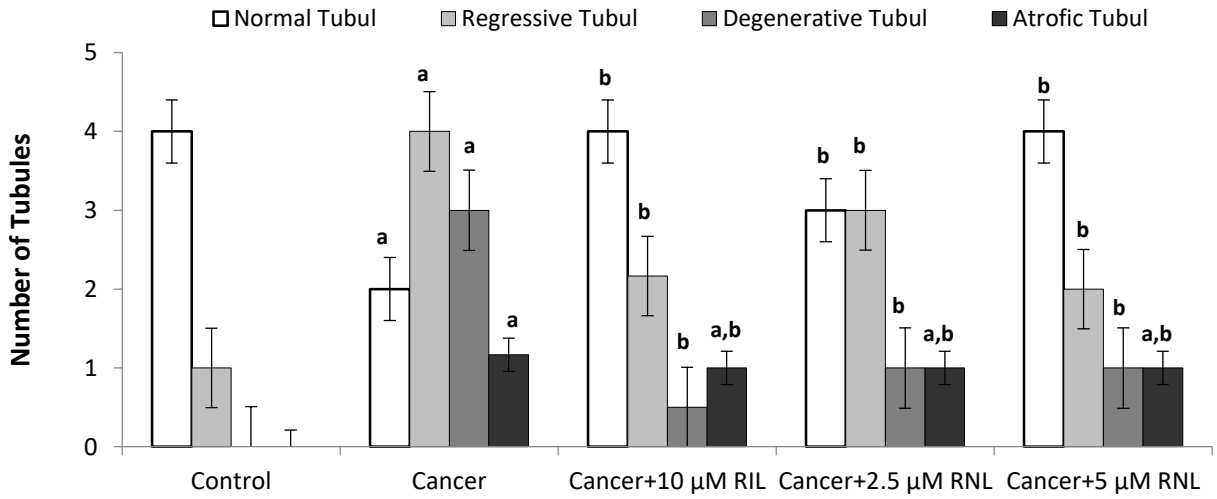

Figure 3. The histopathological scoring of the testes in the experimental groups. ap <0.05, compared to the control group, bp < 0.05, compared to the cancer group.

Histolopathological evaluations and damage score

Testes tissue

When H&E stained testicular sections are examined, in the control group, normal testes' histology was observed (Figure 2A). In the cancer group, degenerated seminiferous tubules, cell debris in the lumen were shown and decreased in the spermatogenic cell line (Figure 2B). The number of degenerative tubules was surged and the spermatogenic cells decreased, and the regressive tubules were also shown in the cancer group. There was a mild disruption in seminiferous tubules, spermatozoa in the lumen, and spermatogenic cells in the cancer + 10 µM RIL, cancer + 2.5 µM RNL, and cancer + 5 µM RNL group. The number of normal tubules was raised in these treatment groups (Figure 2C, D, and E).

The semi-quantitative evaluation of testicular tissue sections made under the light microscope is given in Figure 3. While it was observed that the seminiferous tubule structure was predominantly normal in the control group, a decrease in the number of normal tubules was remarkable in all other groups. The number of regressive tubules and degenerative tubules were found to be increased in the cancer group compared to the control group. The number of normal tubules was diminished while the number of degenerative tubules was increased and there were atrophic tubules in the cancer group compared to the healthy control group. Additionally, the number of normal tubules was increased and the number of regressive, degenerative tubules decreased significantly in cancer + RIL, cancer + RNL groups; the atrophic tubules decreased compared to the cancer group. It was detected that there is a statistically significant difference *($p < 0.05$).*

Liver tissue

When H&E stained liver sections are examined, the control group depicts a normal hepatic parenchymal histology and hepatocyte structure with normal lobular architect and hepatocytes arranged in cords encircling the central canal. (Figure 4A). In the cancer group, pyknotic nucleus, mononuclear cell infiltration, hyperemia, vacuolization, disrupted arrangement of hepatocyte plates, sinusoidal dilatations, and degenerated hepatocytes were observed (Figure 4B). When RIL or RNL treatment was applied, histological appearances showed similarity within the control groups. There was a slight damage in cancer + 10 µM RIL, cancer + 2.5 µM RNL, and cancer + 5 µM RNL groups. Properly hepatocyte arrangement and sinusoidal enlargement were observed (Figure 4C, D, and E).

The semi-quantitative evaluation of liver tissue sections that was assessed under a light microscope is given in Figure 5. There was a significant increase in the cancer groups compared to the control group. RIL and RNL treatment groups were significantly reduced in the liver histological damage compared to the cancer control group *($p < 0.05$).*

Discussion

Within the scientific literature, it was exhibited that the cancer disease caused by other disease mechanisms has a higher mortality rate compared to the sole self-occurring cancer mechanism. Thus, it is predicted that the deaths due to cancer will increase significantly in the coming years. Depending on these reasons, the research to diagnose cancer early on and accurately to prevent and follow up the cancer treatment and its side effects resulting from the cancer treatment gain significance day by day [20].

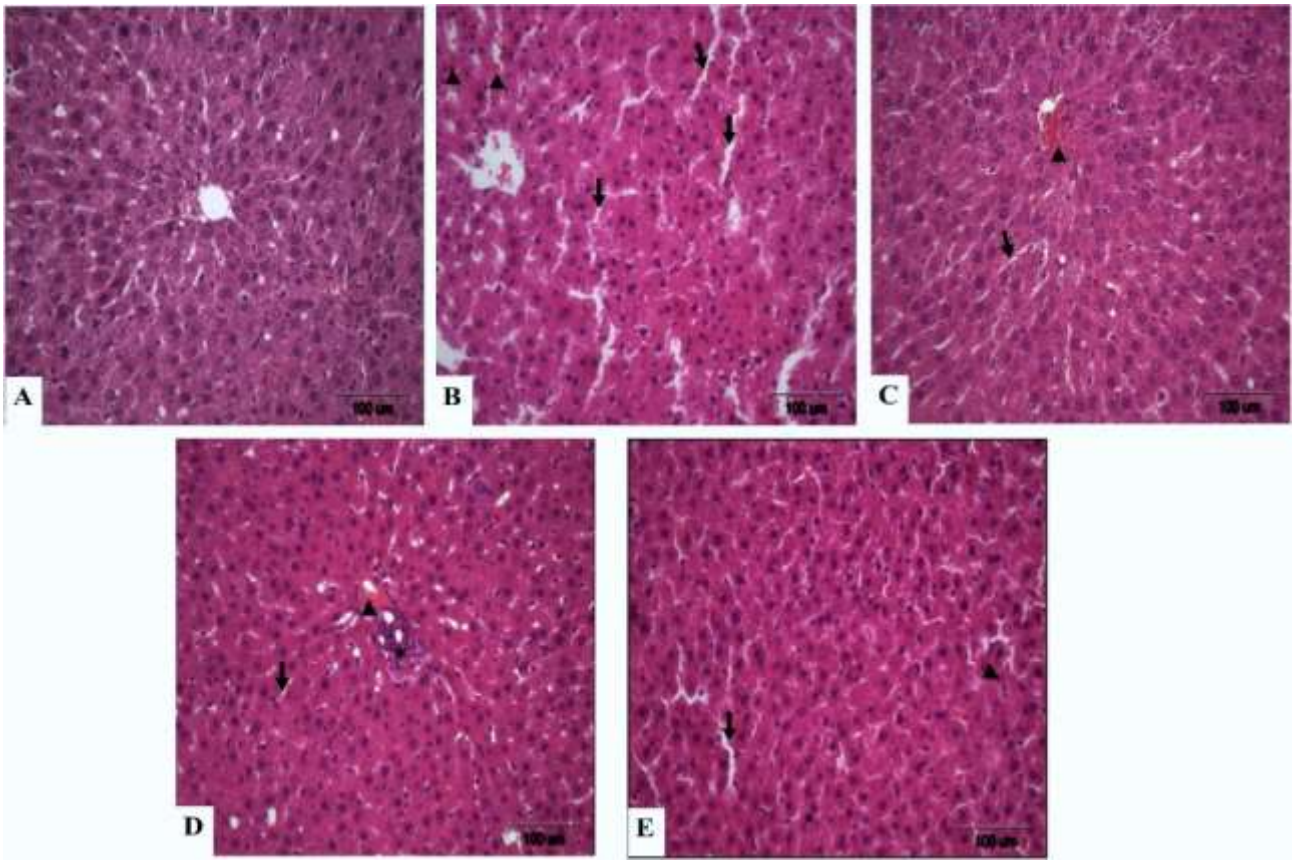

Figure 4. Liver tissue histological appearance: control group (A): cancer group (B), cancer + 10 µM RIL group (C), cancer + 2.5µM RNL group (D), cancer + 5 µM RNL group (E): sinusoidal dilatation (→), hyperemia (►), mononuclear cell infiltration (*), H&E staining, Bar:100 µm, x10 magnification.

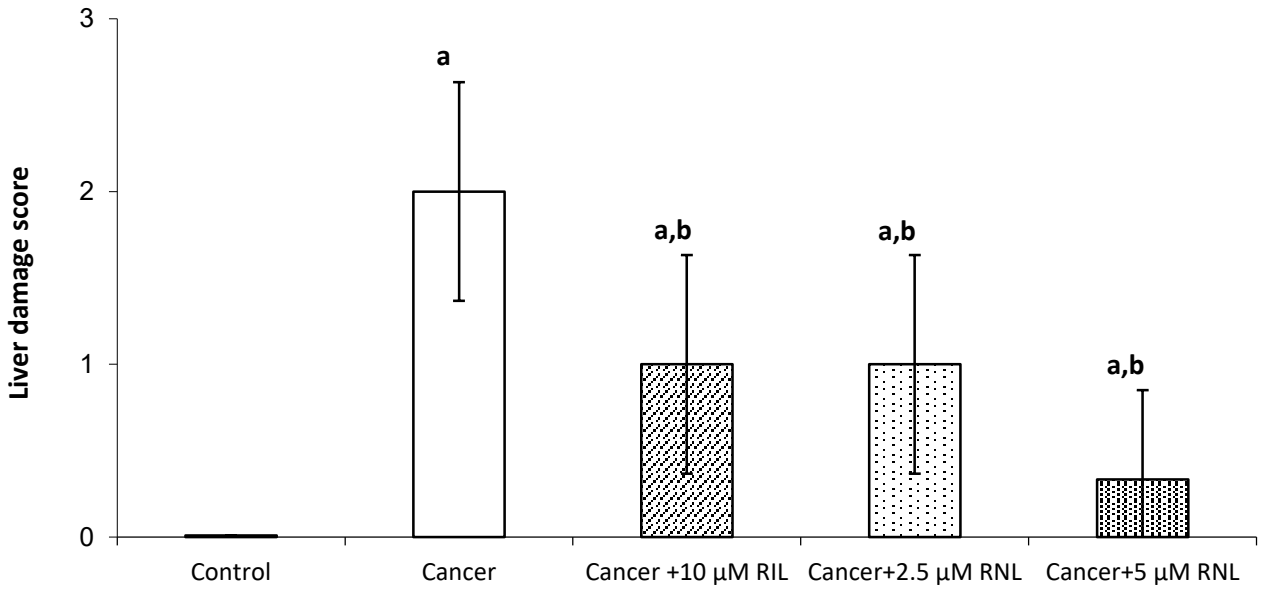

Figure 5. The histopathological scoring of the liver in the experimental groups. ap < *0.05*, compared to the control group, bp < *0.05*, compared to the cancer group.

Although the effects of many drugs or agents in the cancer models have been examined in studies to date, the effects of RIL and RNL treatment on testicular and liver tissue in experimental prostate cancer have not been investigated with an in vivo model before. In our study, we aimed to show the infertility as we anticipated that occurs by the prostate cancer, since the toxicity that it will create in the liver tissue and the therapeutic potential may occur by repositioning of these drugs.

In our study, the primary tumors were observed in about 10 days after subcutaneous inoculation of Mat-LyLu cells into rats [16]. The essential sequels of the systemic administration of RIL and RNL to prostatic tumor-bearing rats were as follows: (1) these treatments mostly prevented testicular and liver damage occurring by the prostate cancer model. (2) In the cancer + 10 μM RIL and cancer + 5 μM RNL group, there was a decrease in the growth of primary tumors insignificantly. Since prostate tissue is not directly affected in the Dunning prostate cancer model, we only evaluated the primary tumor weight for the cancer formation process. The Dunning prostate cancer model was not an orthotopic model, it was administered via subcutaneous injection and the target tissue was not prostate. That's why the first metastasis site was lung tissue in the Dunning model, we discussed the cancer related damage in the testis and liver tissues due to this.

RIL, the only drug approved by the FDA for treating amyotrophic lateral sclerosis, inhibits cancer proliferation through its inhibitory effect on glutamatergic signaling [21, 22]. It has been reported that RIL treatment inhibits the growth of brain tumor. The effects of RIL on these cells have been reported to be related to the inhibition of glucose transporter, a poor prognostic indicator [21]. It demonstrated that RIL inhibits cell growth in breast cancer [23]. According to a different in vitro study, it has been determined that 5 μmol/L RIL (and 20 μmol/L RNL) have anti-invasive effects on MAT-LyLu cells, and suggested RIL (and RNL) may ultimately be "repurposed" as an anti-metastatic drug against PCa [2]. It has been reported that RIL inhibits cell cycle progression and induces apoptosis in tumor cells [24]. The androgen-dependent transcription factor and androgen receptor enhance prostate cancer but inhibiting androgen-dependent or androgen biosynthesis induce remission for only a short time. RIL exerts its anti-tumorigenic effects [25].

RNL, a piperazine derivative sold under the trade name of Ranexa, a selective inhibitor of late sodium current, is a drug used in the treatment of stable angina patients who cannot be adequately controlled with first-line antianginal agents or cannot tolerate these treatments for any reason [26]. RNL has proven to be effective in treating experimental heart failure. It was shown that RNL attenuates trastuzumab-induced heart dysfunction in mice and reduces trastuzumab-induced apoptosis via decreasing the occurrence of caspase-3 fragmentation. RNL limits the production of reactive oxygen species in this experimental model [27]. It was demonstrated that RNL mitigates obesity-induced non-alcoholic fatty liver disease and increases hepatic pyruvate dehydrogenase activity. RNL treatment reverses obesity-induced hepatic steatosis [28]. Matrigel invasion of Mat-LyLu cells and secondary tumorigenesis (in vivo lung metastases) were inhibited by RNL [2,16].

Various mediator molecules of the immune system during the development of cancer, chemotherapeutic drugs used in cancer therapy, and ionizing radiation used in radiotherapy can cause damage to organs such as the liver and testes [10, 11,12]. It suggested that the liver

damage can also be caused by drugs, particularly the anti-tubercular drugs, general anesthetics, paracetamol, and some anti-cancer drugs [29].

The main reason for studying testicular tissue was its close functional association to the prostate, both being parts of the male reproductive system. More so, oxidative stress which is coupled to increased reactive oxygen species and DNA damage in sperm is the leading cause of testicular damage. This situation is directly linked to fertility potentials and can result in infertility [30]. The causes of male-induced fertility problems are cancer and testicular damage. It is known that the prostate tissue has an important contribution to fertility, especially the seminal fluid that provides the transport of sperm at the time of ejaculation [31]. In a case report of a 48 year old patient who was investigated for the metastasis of prostate adenocarcinoma of the prostate, 9 years after the initial diagnosis, prostate specific antigen was gradually elevated and a tumor in the left testicle developed. It has been suggested that testicular metastases may develop, although not frequently, in advanced prostate cancer cases [32].

Prostate cancer cells maintain various stages of the metastasis process through stromal cells, immune cells, and other cells within the tumor microenvironment, as well as cytokines and chemokines secreted by the metastatic site. These proteins exert their effects through autocrine or paracrine mechanisms. The most common site of metastasis for prostate cancer is bone (84% of cases), followed by distant lymph nodes (10.6%), liver (10.2%), and lungs (9.1%). Testicular metastases of primary prostate carcinoma are very rare, ranging from 0.18% to 0.5%, and it is said that their diagnosis can pose great difficulties. The mechanism of metastasis from prostate to testis may vary [33].

To demonstrate the protective effect of the RIL and RNL on testicular damage in rat prostate cancer will be useful in identifying new positive therapeutic options in clinical infertility trials. The histology of the seminiferous tubules in testicular tissues is evaluated as normal, regressive, degenerative, and atrophic [18]. In the present study, the histological evaluation of the testes of rats bearing prostate cancer showed an increase in the abnormal tubules and disorganization. We believe that the histopathological signs of damage observed in the cancer-control group may be an indicator of the development of the infertility process in the prostate cancer model. We can say that the administration of RIL and RNL protects the testicular tissue against infertility which may occur due to the histopathological damage caused by cancer.

The main reason for examining liver tissue is that it is the tissue that we expect to be most affected by toxic damage in the cancer process. The liver is an important organ exposed to attack by reactive oxygen species. The liver is involved in many basic physiological events. Glucose homeostasis, protein, lipid, lipoprotein, and bile acid production, as well as biotransformation, detoxification, conjugation, and excretion of endogenous and exogenous compounds are among the major functions of the liver [34].

There is no information in the literature about liver toxicity in prostate cancer. It was suggested that prostate cancer is a prominent metastatic dormant cancer. It has the worst prognosis when found in the liver compared to other metastatic sites. These metastatic nodules result in an adverse reaction in the pro-metastatic microenvironment; decline, as decided by both dormancy from the nodules name [35]. In our study, there was a significant increase in liver histological damage in cancer-

control groups compared to the healthy control group. RIL and RNL treatment groups were significantly reduced in liver histological damage compared to the cancer-control group. We can say that RIL and RNL application protects the liver tissue which may occur due to the histopathological damage caused by cancer. There are some limitations not studied. This is a part of study, with a small sample size. Animal experiments were planned by considering the minimum number and power analysis that would give a statistically significant result on the grounds of ethical committee obligations. Pharmacological doses were determined based on our previous in vitro findings. In future studies, it is foreseen that the study with different doses and drug combinations will be investigated more extensively with projects with larger budgets.

Conclusions

In conclusion, the effect of RIL and RNL application on testicular and liver damage in the prostate cancer model was first investigated in vivo. Nowadays, the drug repositioning subject has gained attention for researchers and pharmaceutical companies. Drug repositioning involves the investigation of existing drugs for new therapeutic purposes. Some drugs are already in clinical use and can be repurposed as anticancer agents. Especially, some agents have troubling adverse effects that dramatically reduce the life quality of cancer patients, so drug repositioning is a promising strategy [36, 37]. In addition to reducing time and cost, off-label drugs are also a low-risk strategy due to their off-target effects. When comparing repositioning and traditional drug development subject, their safety verified as repositioned drugs passed all clinical tests in Phase I, Phase II, and Phase III. Furthermore, some repositioned agents can be as molecular entities and have more affiliations to be launched once a new indication is discovered [38, 39]. Our microscopic evaluations reveal that RIL and RNL have a protective effect on the testes and liver in the metastatic prostate cancer model. Based on our results, it has been determined that the two drugs (RIL, RNL), which are used for other purposes in the clinic, have a positive effect against the damage to the testes and liver caused by the prostate cancer. The current study could be a model investigation for future work on developing agents for decreasing testicular and liver damage caused by prostate cancer and other types of cancer.

Funding: *This study was supported by TUBITAK (The Scientific and Technological Research Council of Turkey), project number 110T890.*

Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement:

The study was confirmed by Non-Interventional Ethics Committee of University of Istanbul (I.U. HADYEK decision no: 116; date: 2010).

References

[1]Dolfi SC, Medina DJ, Kareddula A, et al. Riluzole exerts distinct antitumor effects from a metabotropic glutamate receptor 1-specific inhibitor on breast cancer cells. Oncotarget. 2017; 8(27):44639-53.

[2]Rizaner N, Uzun S, Fraser SP, et al. Riluzole: Anti-invasive effects on rat prostate cancer cells under normoxic and hypoxic conditions. Basic Clin Pharmacol. 2020;127(4):254-64.

[3]Raghubir M, Nowshin Rahman C, Fang J, et al. Osteosarcoma growth suppression by riluzole delivery via iron oxide nanocage in nude mice. Oncol Rep. 2020;43(1): 169–76.

[4]Hale SL, Shryock JC, Belardinelli L, et al. Late sodium current inhibition as a new cardioprotective approach. J Mol Cell Cardiol. 2008;44(6):954-67.

[5]Antzelevitch C, Nesterenko V, Shryock JC, et al. The role of late I Na in development of cardiac arrhythmias. Handb Exp Pharmacol. 2014; 221:137-68.

[6]Nelson M, Yang M, Millican-Slater R, et al. Nav1.5 regulates breast tumor growth and metastatic dissemination in vivo. Oncotarget. 2015;6 (32):32914–29.

[7]Bugan I, Kucuk S, Karagoz Z, et al. Anti-metastatic effect of ranolazine in an in vivo rat model of prostate cancer, and expression of voltage-gated sodium channel protein in human prostate. Prostate Cancer Prostatic Dis. 2019; 22 (4):569-79.

[8]Ajroud-Driss S, Saeed M, Khan H, et al. Riluzole metabolism and CYP1A1/2 polymorphisms in patients with ALS. Amyotroph Lateral Scler. 2007; 8(5):305-309.

[9]Cheng JW. Ranolazine for the management of coronary artery disease. Clinical Therapeutics. 2006; 28(12):1996-2007.

[10]Thu YM, Richmond A. NF-κB inducing kinase: a key regulator in the immune system and in cancer. Cytokine Growth Factor Rev. 2010;21(4):213-26.

[11]Valiño-Rivas L, Vaquero JJ, Sucunza D, et al. NIK as a Druggable Mediator of Tissue Injury. Trends Mol Med. 2019; 25 (4):341-60.

[12]Farhood B, Khodamoradi E, Hoseini-Ghahfarokhi M, et al. TGF-β in radiotherapy: mechanisms of tumor resistance and normal tissues injury. Pharmacol Res. 2020; 155:104745.

[13]Guth A, Monk E, Agarwal R, et al. Targeting Fat Oxidation in Mouse Prostate Cancer Decreases Tumor Growth and Stimulates Anti-Cancer Immunity. Int J Mol Sci. 2020; 21(24): 9660.

[14]Pienta KJ, Abate-Shen C, Agus DB, et al. The current state of preclinical prostate cancer animal models. Prostate. 2008;68 (6):629-39.

[15]Tennant TR, Kim H, Sokoloff M, et al. The Dunning model, Prostate. 2000; 43 (4):295-302.

[16]Bugan I, Karagoz Z, Altun S, et al. Gabapentin, an analgesic used against cancer-associated neuropathic pain: Effects on prostate cancer progression in an in vivo rat model. Basic Clin Pharmacol Toxicol. 2016; 118(3): 200–207.

[17]Wang T, Huang W, Cheng F. Baclofen a GABAB receptor ago- 679 nist, inhibits human hepatocellular carcinoma cell growth in vitro 680 and in vivo. Life Sci. 2008; 82 (9-10):536–41.

[18]Hess RA, Linder RE, Strader LF, et al. Acute effects and long term sequelae of 1,3-dinitrobenzene on male reproduction in the rat II. quantitative and qualitative histopathology of the testis. J Androl. 1988; 9(5): 327- 42.

[19] Bhattacharya A, Dhar P, Mehra RD. Preliminary morphological and biochemical changes in rat liver following postnatal exposure to sodium arsenite. Anat Cell Biol. 2012; 45 (4): 229-40.

[20] Siegel RL, Miller KD, Jemal A. Cancer statistics, CA: Cancer J. Clin. 2019; 69(1):7-34.

[21] Sperling S, Aung T, Martin S, et al. Riluzole: a potential therapeutic intervention in human brain tumor stem-like cells. Oncotarget. 2017; 20(57):96697-96709.

[22] Taguchi YH, Wang H. Genetic Association between Amyotrophic Lateral Sclerosis and Cancer. Genes (Basel). 2017; 8(10):243.

[23] Speyer C, Nassar MA, Hachem AH, et al. Riluzole mediates anti-tumor properties in breast cancer cells independent of metabotropic glutamate receptor 1. Breast Cancer Res Treat. 2016; 157:217-28.

[24] Lemieszek MK, Stepulak A, Sawa-Wejksza K, et al. Riluzole Inhibits Proliferation, Migration and Cell Cycle Progression and Induces Apoptosis in Tumor Cells of Various Origins. Anti-Cancer Agents Med Chem. 2018;18 (4):565-72.

[25] Wadosky KM, Shourideh M, Goodrich DW, et al. Riluzole induces AR degradation via endoplasmic reticulum stress pathway in androgen-dependent and castration-resistant prostate cancer cells. Prostate. 2019;79 (2):140-150.

[26] Rayner-Hartley E, Sedlak T. Ranolazine: A Contemporary Review. J Am Heart Assoc. 2016;5(3):e003196.

[27] Riccio G, Antonucci S, Coppola C, et al. Ranolazine Attenuates Trastuzumab-Induced Heart Dysfunction by Modulating ROS Production. Front physiol. 2018; 9:38.

[28] Al Batran R, Gopal K, Aburasayn H, et al. The antianginal ranolazine mitigates obesity-induced nonalcoholic fatty liver disease and increases hepatic pyruvate dehydrogenase activity. JCI Insight. 2019; 4 (1): e124643.

[29] Teschke R. Hepatotoxicity: Molecular Mechanisms and Pathophysiology. Int J Mol Sci. 2019; 20 (1): 211.

[30] Smith R, Kaune H, Parodi D, et al. Increased sperm DNA damage in patients with varicocele relationship with seminal oxidative stress. Hum Reprod. 2006; 21(4): 986–993.

[31] Hoppe BS, Harris S, Rhoton-Vlasak A, et al. Sperm preservation and neutron contamination following proton therapy for prostate cancer study. Acta Oncol. 2017;56(1):17-20.

[32] [32] Campara Z, Simic D, Aleksic P, et al. Metastasis of prostate adenocarcinoma to the testis. Med Arch. 2016; 70(4): 318-20.

[33] Raghuvanshi K, Deshmukh H, Raval A, et al. Cancer prostate metastasis to testis: A rare encounter, 2018; 1(2): 94-96.

[34] Vdoviaková K, Vdoviaková K, Petrovová E, et al. Importance rat liver morphology and vasculature in surgical research. Med Sci Monit. 2016; 22: 4716-28.

[35] Ma B, Wells A, Wei L, et al. Prostate cancer liver metastasis: Dormancy and resistance to therapy, Seminars in Cancer Biology. 2021; 71: 2-9.

[36] Biber A, Durusu IZ, Özen C. In vitro anticancer effect of tricyclic antidepressant nortriptyline on multiple myeloma. Turk J Biol. 2018; 42 (5):414-21.

[37] Turanli B, Grøtli M, Boren J, et al. Drug Repositioning for Effective Prostate Cancer Treatment. Front physiol. 2018; 9:500.

[38] Xue H, Li J, Xie H, et al. Review of Drug Repositioning Approaches and Resources. Int J Biol Sci. 2018;14 (10):1232-44.

[39] Ozdemir A, Ark M. A novel ROCK inhibitor: off-target effects of metformin. Turk J Biol. 2021; 45 (1):35-45.