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Original Article

Losartan, an angiotensin-II type 1 receptor blocker, attenuates CCl_4-induced liver fibrosis with a positive impact on survival in mice

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ABSTRACT

Background: Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin-II receptor blockers (ARBs) may provide synergistic effects to existing chemotherapies by reducing angiotensin II-mediated angiogenesis, fibrogenesis, mitogenesis and oxidative stress.
Objective: We aimed to examine the effect of the ARB, losartan and the ACEIs, perindopril and fosinopril on carbon tetrachloride (CCl4)-induced liver fibrosis on the histopathologic level and assess their impact on survival of mice.
Methods: liver fibrosis was induced by CCl4 and examined histologically. Mice were treated with silymarin (SI) (30 mg/kg), perindopril (PE) (1 mg/kg), fosinopril (FO) (2 mg/ kg) or losartan (LO) (10 mg/kg). Cumulative survival was done using the Kaplan-Meier method and the log-rank test.
Results: The administration of PE and FO resulted in improved liver histology without survival benefits to mice. However, losartan demonstrated marked improvement of liver histology and a positive impact on survival which was comparable to silymarin.
Conclusion: Interfering the renin-angiotensin system (RAS) through the blockade of angiotensin-II type 1 (AT1) receptors improved liver histology of CCl4-induced hepatic fibrosis that was associated with longer survival in mice.
Keywords: CCl4; liver fibrosis; renin-angiotensin system; survival analysis

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INTRODUCTION

Hepatic fibrosis is the common histologic feature that represent the pathologic base in most liver diseases that ultimately leads to cirrhosis and its consequence of hepatocellular carcinoma (HCC) [1]. Various etiologies include viral, alcoholic, non-alcoholic steatohepatitis (NASH), autoimmune, and metabolic disease. Liver cirrhosis is characterized by excessive deposition of the extracellular matrix (ECM) [2]. Currently, there is no effective therapy for treatment of liver fibrosis in which the causative agent cannot be removed [3] that results in bad prognosis and decreased overall survival.

Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), having well established safety profiles and low economic cost, may provide synergistic effects to existing chemotherapies by reducing angiotensin-II-mediated angiogenesis, fibrogenesis, mitogenesis, metastasis and oxidative stress. The renin-angiotensin system (RAS) plays an important role in controlling liver fibrosis [4]. It is well known that the RAS influences the progression of many chronic liver diseases [5, 6]. Furthermore, RAS modulation to treat liver fibrosis by ARB has been reported experimentally [7].

The mechanistic pathways of fibrosis involves an increase in hepatic transforming growth factor β1 (TGF-β1) and pro-inflammatory cytokine levels that were attenuated in angiotensin-II type 1 receptor (AT1R) knockout mice compared to wild type (WT) mice [8]. In addition, Bataller, Schwabe [9] demonstrated that systemic angiotensin-II (Ang-II) augments liver fibrosis. Moreover, It was reported that ACEIs increase overall survival (OS) in patients with renal cell, pancreatic, brain, and lung cancer [10, 11].

None is known about the impact of Ang-II inhibition on the OS of mice with chemically induced liver fibrosis. In addition, abundant experimental evidence represents an attractive antifibrotic target that encourages evaluation of the RAS inhibition in the injured liver and assessing its impact on survival in case of persistent causative agent. Therefore, the objective of the current study was directed to examine potential benefits of Ang-II inhibition on the histologic level and to assess the association between Ang-II inhibitors and survival. A model of repetitive administration of carbon tetrachloride (CCl4) was used to represent advanced liver fibrosis with persistent causative agent. The effects of RAS inhibitors were compared with respect to silymarin.

MATERIALS AND METHODS

Animals: A total of one hundred 4-6-week-old male Swiss albino mice of CD-1 strain weighing 18-20 g were used in the current experiment. They were supplied from the unit of schistosome biologic materials supply program, Theodor Bilharz Research Institute (SBSP-TBRI) and housed in polycarbonate cages in accordance with the National Institute of Health guide for the care and use of laboratory animals, Egypt. All experimental procedures were approved by local authorities at TBRI. All of the mice were fed rodent chow (23 % protein and 4 % fat) and received water ad libitum. They were kept under standard laboratory conditions of (21 C^o, 45-55% humidity) and exposed to a 12:12 light dark cycle. The animals were acclimatized for 1 week prior to the experiment.

Drugs and chemicals: CCl4 was purchased from Sigma Aldrich (St. Louis, MO, USA). SI, PE, FO, and LO were provided by Bayer AG (Berlin, Germany), Servier (Suresnes, France), Bristol-Myers Squibb (New York, NY, USA), and Merck (Kenilworth, NJ, USA), respectively.

Experimental design: Mice were randomly allocated to six groups. Group 1 (Normal): mice received the vehicle (olive oil) only (n = 15). Group 2 (CCl4): mice received i.p. injections of CCl4 at a dose of (1 ul/gm mouse) twice/week (n = 25). Groups 3 (SI), 4 (PE), 5 (FO), and 6 (LO) (n = 15 of each): mice received CCl4 plus SI (30 mg/kg), CCl4 plus PE (1 mg/kg), CCl4 plus FO (2 mg/kg), and CCl4 plus LO (10 mg/kg), respectively. The drugs were administered once daily by oral gavage starting from the 1st day and continued till the end of the experimental period for up to 5 weeks.

Rationale of drug dosing: The dose of CCl4 was selected in consistence with that defined by Constandinou, Henderson [12], and was diluted (1:2.5 v/v) in olive oil while the other drugs were freshly prepared immediately before use by suspending in distilled water. The equivalent mouse doses of silymarin, perindopril, fosinopril, and losartan were calculated by interpolating from the corresponding lowest effective human dose using approximate dose conversion factors described by Freireich et al. [13].

Histopathological examination: After decapitation, liver tissues from mice groups were fixed in 10% buffered formalin. After embedding in paraffin, sections of about 5 µm thick from the paraffin blocks were stained with hematoxylin and

eosin (H&E) and Masson trichrome stains for histologic examination.

Statistical analysis: Statistical analysis was performed using GraphPad prism software version 6 (GraphPad Software Inc., La Jolla, CA, USA). For cumulative survival, the log-rank (Mantel-cox) test was used for assessing significance of difference between groups in the Kaplan-meier analysis. P values < 0.05 were considered significant.

RESULTS

Histopathological examination: Representative histological appearance of liver specimens from untreated normal control mice (fig. 1a) showed hepatic lobules with intact lobular architecture. Liver cells are arranged in cords of one to two cell-thick, radiating from a central vein towards the lobular periphery with blood sinusoids in-between. Hepatocytes are polyhedral with abundant granular eosinophilic cytoplasm and one spherical nuclei with dispersed chromatin. Portal tracts are of normal shape and thickness. Liver sections from the CCl4-treated mice (fig. 1b) showed disorganized architecture with extensive fibrous tissue deposition around central veins and portal tracts as well as into hepatic lobules. Fibrosis, score (3-4) according to scoring system described by Bedossa [14], links portal tracts to central veins and to other portal tracts forming centro-portal and portal-portal bridging fibrosis. Hepatic parenchyma showed congested vessels and sinusoids, in addition to intra-lobular inflammatory infiltrate of lymphocytes and plasma cells along with foci of cholestasis, hepatocellular degeneration and necrosis. The administration of SI (fig. 1c), PE (fig. 1d), FO (fig. 1e), or LO (fig. 1f) improved the histological picture of liver showing reduction of fibrosis, score (1-2).

Survival analysis: Kaplan-Meier survival curves depicted in Fig. 2 (a) revealed that CCl4-treated mice had a higher mortality rate vs silymarin treated mice that demonstrated higher survival probability and significant survival curves (log rank test p= 0.01, hazard ratio = 5.07). The administration of perindopril (fig. 2b), fosinopril (fig. 2c) resulted in higher cumulative survival vs CCl4-treated mice. However, survival curves were non-significant (log rank test p= 0.07, hazard ratio = 2.9) and (log rank test p= 0.24, hazard ratio = 1.88), respectively. On the other hand, losartan treated mice (fig. 2d) resulted in significant survival curves vs CCl4-treated mice and showed higher survival proportions (log rank test p= 0.02, hazard ratio = 4.66).

The administration of perindopril (fig. 3a), fosinopril (fig 3b) and losartan (fig 3c) demonstrated non-significant survival curves vs silymarin (log rank test p= 0.33, 0.26 and 0.6), respectively.

Log-rank analysis for comparison of survival revealed no significant differences in the lifespan of mice among treatment groups (fig 4a, log rank test p= 0.7) and significant differences among CCl4-treated mice (fig 4b, log rank test p= 0.04).

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DISCUSSION

The discovery of new therapeutic interventions or repurposing current medications is vital to improve the survival and prognosis of fibrosis/cirrhosis patients. RAS inhibition is a prospective interesting target for the prevention of hepatic fibrosis. Therefore, we aimed to determine whether RAS inhibitors improve the overall survival of mice with hepatic fibrosis induced by repetitive administration of CCl4 making a direct comparison for their effects with silymarin on the histologic level. Repetitive administration makes a representation of persistent causative agent.

In vitro studies have shown that Ang-II is a mitogenic protein for hepatic stellate cells (HSCs). In the healthy liver, HSCs do not express AT1Rs nor do they produce Ang-II. However, following chronic liver injury, activated HSCs and myofibroblasts have the ability to both express AT1Rs and generate Ang-II which exerts an array of pro-inflammatory and profibrogenic actions including TGF-β1 expression upregulation [15, 16] that can be reversed by losartan [17].

Pinter, Weinmann [18] found that in HCC patients, RAS inhibition had a better median overall survival (OS) (19.5 mo) compared to those treated with either sorafenib (10.9 mo) or RAS inhibition (9.7 mo) alone (p= 0.043). Another observation study suggested that ARBs treatment during erlotinib treatment may prolong OS of metastatic non-small cell lung cancer (NSCLC) patients [19]. However, none is known about the impact of RAS inhibition on survival in the setting of liver fibrosis/cirrhosis when the causative agent is persistent.

In the present study, the selection of perindopril (a high tissue affinity ACEI) and fosinopril (a low tissue affinity ACEI) [20, 21] was intentional to value the concept of class effect regarding therapeutic efficacy of ACEIs [22, 23]. Moreover, we evaluated the effect of inhibiting RAS signaling through blocking AT1 receptors using the ARB, losartan.

Hepatic fibrosis in CCl4-treated mice was evident on the histologic level. The histologic picture of hepatic fibrosis in mice treated with CCl4 was improved after treatment with the selected ACEIs, perindopril and fosinopril and the ARB, losartan. This was manifested as a regression of fibrotic and inflammatory changes from grade (3-4) to grade (1-2) indicating amelioration of hepatic fibrosis in short term treatment with the used drugs.

The lifespan of CCl4-treated mice was significantly decreased compared with normal mice that showed no mortality. This is consistent with CCl4-induced systemic toxicity. Ten days post-induction of hepatic fibrosis, the lifespan of mice was started to significantly increase by losartan treatment compared with CCl4-treated mice parallel to marked improvement in liver histology. This was evident by regression of fibrosis score and restoration of lobular architecture. Despite histologic improvement, perindopril did not significantly increase survival of mice. However, a trend of survival prolongation is established, p= 0.07. The discrepancy in the results between ACEIs in the present study might be consistent with the high tissue affinity of perindopril compared with fosinopril (low tissue affinity ACEI) that improved histologic picture to a certain extent with no survival benefits, p= 0.24.

The current study introduce some interesting findings. First, the histologic picture of liver tissues was improved upon treatment with perindopril, fosinopril, or losartan as monotherapy and was comparable to that of silymarin at their lowest effective doses. Second, interfering the RAS either through the inhibition of ACE or the blockade of AT1Rs has almost the same histologic benefit. However, losartan seems more promising because losartan-treated mice showed higher survival probability. However, perindopril needs further evaluation with changing doses and/or therapy duration. Third, the tissue affinity of the ACEIs might has a positive impact on its hepatoprotective effect in this model of hepatic fibrosis and needs further investigation.

In conclusion, the present results provide a potential for the therapeutic benefits of ACEIs or ARBs in liver tissues suggesting the prospective use of ACEIs or ARBs in managing patients with fibrosis/cirrhosis either as monotherapy or in combination with other agents. Furthermore, losartan is the most promising agent with positive impact on survival of mice suggesting an advantage of ARBs over ACEIs as new hepatoprotective agents in drug repositioning strategies.

Conflict of interest: The authors declare no conflict of interest.

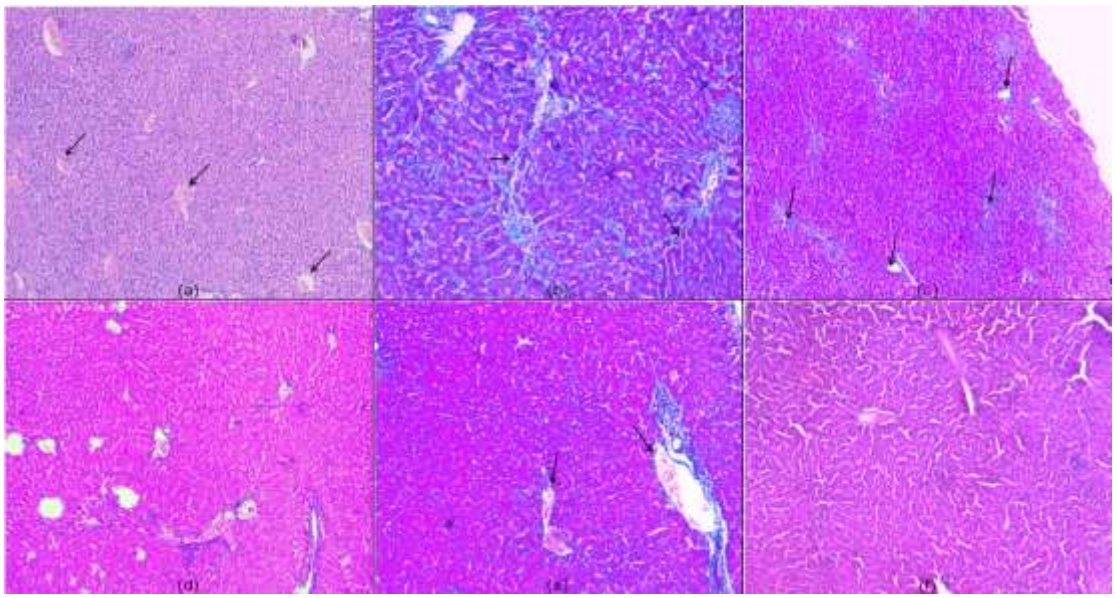

Fig. 1: Representative light micrographs of liver specimens from: **(a)** normal untreated control mice showing liver histology characterized by normal radial arrangement of hepatocyte rays around central veins (arrows) that are separated by sinusoids (H&E x100), **(b)** CCl4-treated mice showing fibrous tissue deposition around blood vessels (arrows) (Masson trichrome x200), **(c)** silymarin treated mice showing fibrotic tissue that is reduced in extent and is limited to deposition around blood vessels (arrows) (Masson trichrome x100), **(d)** perindopril treated mice showing restoration of lobular architecture and reduced extent of inflammation (H&E x100), **(e)** fosinopril treated mice showing restoration of lobular architecture and fibrosis is limited to traces around vessel walls (arrows) (Masson trichrome x200), **(f)** losartan treated mice showing restoration of lobular architecture, disappearance of inflammation and fibrosis (H&E x100).

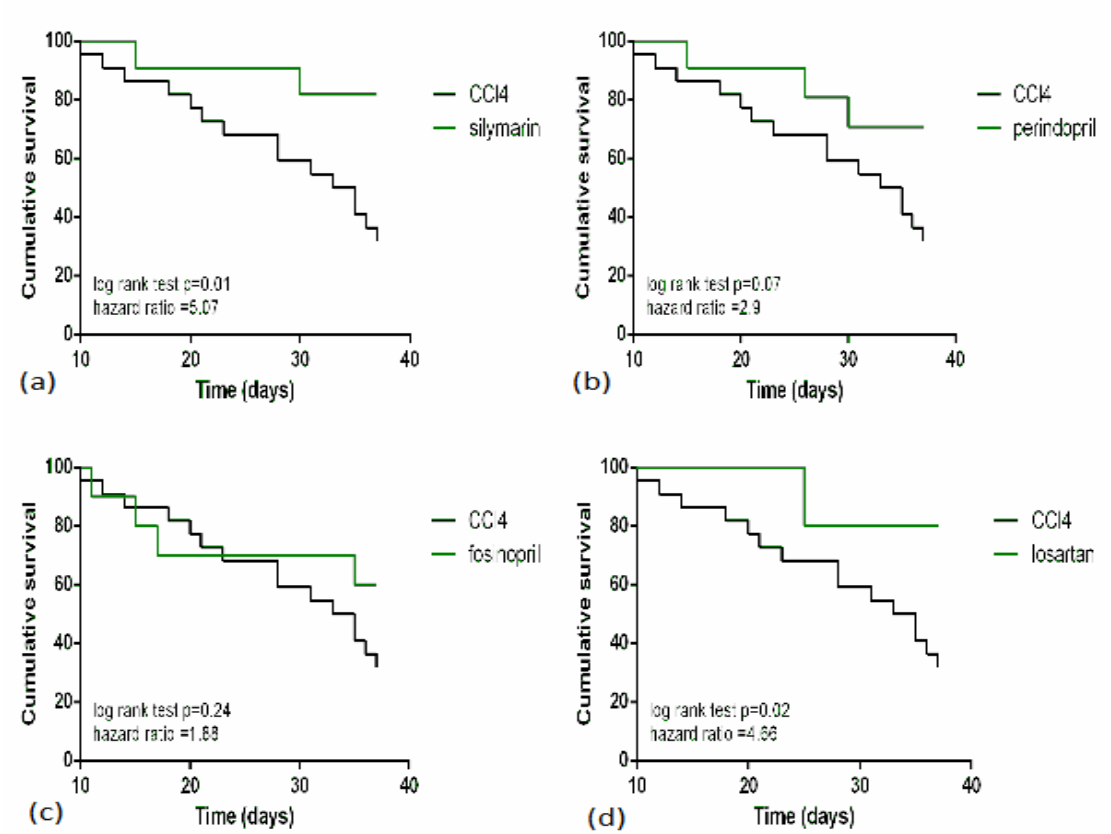

Fig. 2: Kaplan-Meier survival curves of **(a)** silymarin vs CCl4, **(b)** perindopril vs CCl4, **(c)** fosinopril vs CCl4, **(d)** losartan vs CCl4. Statistical analysis was performed using log-rank test (Mantel-cox method). P values < 0.05 were considered significant

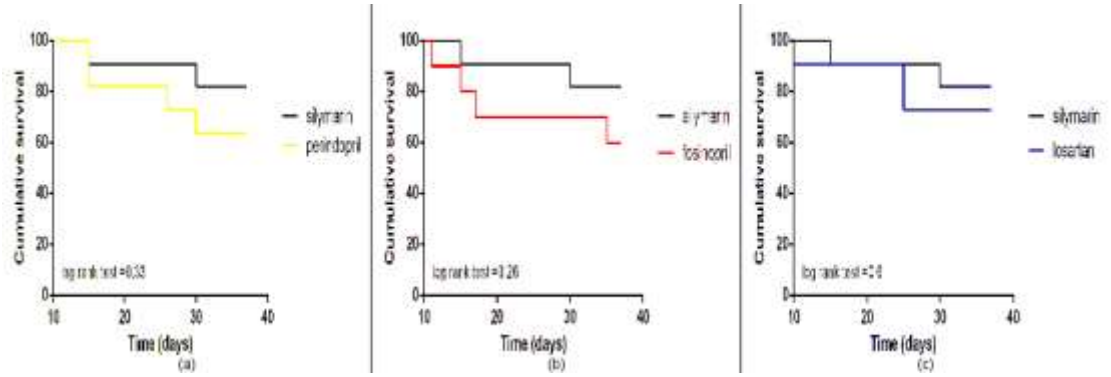

Fig. 3: Kaplan-Meier survival curves of **(a)** perindopril vs silymarin, **(b)** fosinopril vs silymarin, **(c)** losartan vs silymarin. Statistical analysis was performed using log-rank test (Mantel-cox method). P values < 0.05 were considered significant.

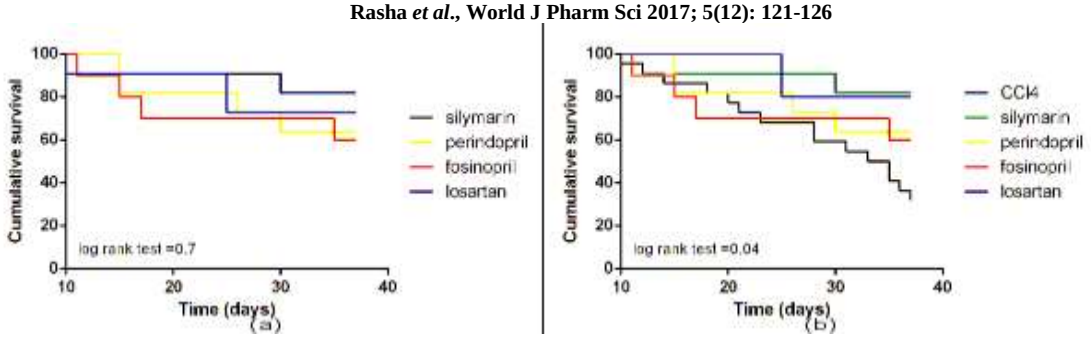

Fig. 4: Kaplan-Meier survival curves of **(a)** cumulative survival among treatment groups, **(b)** cumulative survival among treatment groups and CCl4. Statistical analysis was performed using log-rank test (Mantel-cox method). P values < 0.05 were considered significant.

REFERENCES

1. Sakurai, T. and M. Kudo, *Molecular link between liver fibrosis and hepatocellular carcinoma.* Liver cancer, 2013. **2**(3-4): p. 365-366.
2. Jiao, J., et al., *Hepatic fibrosis.* Current opinion in gastroenterology, 2009. **25**(3): p. 223.
3. Arthur, M.J., *Reversibility of liver fibrosis and cirrhosis following treatment for hepatitis C.* Gastroenterology, 2002. **122**(5): p. 1525-1528.
4. Pereira, R.M., et al., *The renin–angiotensin system in a rat model of hepatic fibrosis: evidence for a protective role of angiotensin-(1–7).* Journal of hepatology, 2007. **46**(4): p. 674-681.
5. Lubel, J.S., et al., *Angiotensin-(1–7), an alternative metabolite of the renin–angiotensin system, is up-regulated in human liver disease and has antifibrotic activity in the bile-duct-ligated rat.* Clinical Science, 2009. **117**(11): p. 375-386.
6. Bataller, R., et al., *Liver fibrogenesis: a new role for the renin–angiotensin system.* Antioxidants & redox signaling, 2005. **7**(9-10): p. 1346-1355.
7. Moreno, M., et al., *Reduction of advanced liver fibrosis by short-term targeted delivery of an angiotensin receptor blocker to hepatic stellate cells in rats.* Hepatology, 2010. **51**(3): p. 942-952.
8. Yi, E.-t., et al., *Telmisartan attenuates hepatic fibrosis in bile duct-ligated rats.* Acta Pharmacologica Sinica, 2012. **33**(12): p. 1518-1524.
9. Bataller, R., et al., *NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis.* Journal of Clinical Investigation, 2003. **112**(9): p. 1383.
10. Keizman, D., et al., *Angiotensin system inhibitors and outcome of sunitinib treatment in patients with metastatic renal cell carcinoma: a retrospective examination.* European Journal of Cancer, 2011. **47**(13): p. 1955-1961.
11. Menter, A.R., et al., *Effect of Angiotensin System Inhibitors on Survival in Patients Receiving Chemotherapy for Advanced Non–Small-Cell Lung Cancer.* Clinical lung cancer, 2017. **18**(2): p. 189-197. e3.
12. Constandinou, C., et al., *Modeling liver fibrosis in rodents.* Methods Mol Med, 2005. **117**: p. 237-50.
13. Freireich, E.J., et al., *Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man.* Cancer Chemother Rep, 1966. **50**(4): p. 219-44.
14. Bedossa, P., *Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C.* Hepatology, 1994. **20**(1): p. 15-20.
15. Wei, H.S., et al., *The regulatory role of AT1 receptor on activated HSCs in hepatic fibrogenesis: effects of RAS inhibitors on hepatic fibrosis induced by CCl4.* World journal of gastroenterology, 2000. **6**(6): p. 824.
16. Bataller, R., et al., *Activated human hepatic stellate cells express the renin-angiotensin system and synthesize angiotensin II.* Gastroenterology, 2003. **125**(1): p. 117-125.
17. Leung, P.S., et al., *Expression and localization of AT 1 receptors in hepatic Kupffer cells: its potential role in regulating a fibrogenic response.* Regulatory peptides, 2003. **116**(1): p. 61-69.
18. Pinter, M., et al., *Use of inhibitors of the renin–angiotensin system is associated with longer survival in patients with hepatocellular carcinoma.* United European Gastroenterology Journal, 2017: p. 2050640617695698.
19. Aydiner, A., et al., *Renin-Angiotensin system blockers may prolong survival of metastatic non-small cell lung cancer patients receiving erlotinib.* Medicine, 2015. **94**(22).
20. Fabris, B., et al., *Characterization of cardiac angiotensin converting enzyme (ACE) and in vivo inhibition following oral quinapril to rats.* Br J Pharmacol, 1990. **100**(3): p. 651-5.
21. Sauer, W.H., et al., *Class effect of angiotensin-converting enzyme inhibitors on prevention of myocardial infarction.* Am J Cardiol, 2004. **94**(9): p. 1171-3.
22. Furberg, C.D., et al., *Are drugs within a class interchangeable?* Lancet, 1999. **354**(9185): p. 1202-4.
23. Lala, A. and M.A. McLaughlin, *Do ACE inhibitors all provide the same outcomes benefits in high-risk cardiovascular patients?* Curr Hypertens Rep, 2008. **10**(4): p. 286-92.