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Original Article

Itraconazole ameliorates diethylnitrosamine-induced hepatocellular carcinoma in mice

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ABSTRACT

Introduction: Hepatocellular carcinoma is a major cause of morbidity and mortality worldwide. Sorafenib as a multi kinase inhibitor prolongs patients' survival for only a few months. Therefore, there is a great need for alternatives. It was proposed that itraconazole is a powerful inhibitor of angiogenesis and hedgehog signaling.
Aim of the study: we aimed to examine the effect of itraconazole (300 mg/kg) administered orally as a monotherapy or as adjunct therapy with sorafenib (30mg/kg) on diethylnitrosamine-induced HCC in mice.
Methods: Five micron serial sections from paraffin blocks were stained with hematoxylin and eosin. Slides were located in Mayer's Hematoxylin dye for three minutes followed by rinsing with distilled water. Then placed in Eosin dye for two minutes followed by rinsing with distilled water and three baths of alcohol with decreasing concentrations (80%-95%-100%) of three minutes each and two baths of xylene for another three minutes.
Results: The administration of itraconazole demonstrated improved liver histology and demonstrated regression of tumor production rates that was linked to attenuation of fibrosis and inflammatory cell infiltration in addition to restoration of lobular architecture.
Conclusion: These results suggest that itraconazole can serve as promising candidate in the management of HCC and further studies are warranted to investigate the underlying mechanisms behind its anti-tumor activity.

Keywords: Hepatocellular carcinoma; sorafenib; itraconazole

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INTRODUCTION

Hepatocellular carcinoma is one of the prevalent global cancers [1] that is associated with a change in the angiogenesis behavior and proliferation [2]. The first-choice therapy for HCC patients is the multikinase inhibitor, sorafenib [3-11]. However, it has the ability of only increasing the patients' survival for a few months. Therefore, a great demand exists to find new active therapies for the management of HCC [4]. Itraconazole, a benz-imidazole antifungal drug, is clinically administered for a long time and its safety profile has been well recognized [12]. Many studies proposed that it is a powerful inhibitor of several types of carcinoma [13]. However, its anti-tumor activity on hepatocellular carcinoma still needs to be investigated. In HCC, vascular endothelial growth factor is implicated in neovascularization and tumor cell proliferation [14]. Therefore, it represents a significant target by exploring novel anti-angiogenic agents. A recent study has shown that benzimidazole derivatives overwhelm vascular endothelial growth factor in cancer cells [15]. Therefore, the objective of this study is to investigate the effect of itraconazole as a monotherapeutic agent or as adjuvant therapy with sorafenib on diethylnitrosamine-induced HCC in mice and to evaluate the anti-tumor activity of itraconazole on histopathological basis.

MATERIALS AND METHODS

Animals: One hundred and fifty male CD-1 Swiss albino mice (21-day-old, 15-20 g) obtained from biologic materials supply program, Theodor Bilhars Research Institute, Giza, Egypt were used in this study. Animals were fed rodent chow (4 % fat and 23 % protein), received water ad libitum and were kept under standard conditions (21 Co, 45-50% humidity). Mice were allowed to acclimate for 1 week prior to commencing experiments.

Ethical statement: Mice were maintained according to the National Institute of Health guidelines for the care and use of laboratory animals. All experimental procedures followed the guidelines of Research Ethics Committee at the Faculty of Pharmacy, Delta University for Science and Technology.

Drugs and chemicals: Diethylnitrosamine was provided from Sigma Aldrich (St. Louis, MO, USA). Sorafenib and itraconazole were supplied from Bayer AG (Berlin, Germany), Global Napi Pharmaceuticals (Egypt), respectively.

Experimental design: Mice were allocated into five experimental groups (n= 15-20): (Normal), mice were administered I.P. injection of saline solution once a week for 16 weeks. (diethylnitrosamine), mice were administered I.P. injections of diethylnitrosamine once a week for 16 weeks. (sorafenib 30 mg/kg/day P.O.) starting the 60th day post induction of hepatocellular carcinoma as described. (itraconazole 30 mg/kg/day P.O.) starting the 60th day post induction of hepatocellular carcinoma as described. (sorafenib 30 mg/kg/day P.O. + itraconazole 30 mg/kg/day P.O.) starting the 60th day post induction of hepatocellular carcinoma as described.

Rationale of drug dosing: Genetically, the diethylnitrosamine -model is a good demonstration of hepatocellular carcinoma that is associated with bad prognosis [7]. One % diethylnitrosamine solution in normal saline was prepared and itraconazole was used as a suspension in distilled water. Diethylnitrosamine schedule of administration was modified as following: first week (30 mg/ kg), second week (50 mg/kg), third week (50 mg/kg), fourth week (70 mg/kg), fifth week (100 mg/kg), sixth-sixteenth weeks (50 mg/kg)[16]. The dose of sorafenib was chosen according to a previous study [17]. The dose of itraconazole was selected based on its clinical dose.

Methods

Tissue sampling: Sixteen weeks post induction of hepatocellular carcinoma, mice were euthanatized by decapitation. Livers were isolated and washed with ice-cold phosphate buffered saline (0.01 mol/L, pH 7.2) and then fixed in 10 % buffered formalin, pH 7, at 25°C for histo-pathological examination.

Histologic examination: Liver tissues were kept in paraffin. Five micron serial sections from paraffin blocks were stained with hematoxylin and eosin (H&E) for histopathological examination as follows: Slides were located in Mayer's Hematoxylin dye for three minutes followed by rinsing with distilled water. Then placed in Eosin dye for two minutes followed by rinsing with distilled water and three baths of alcohol with decreasing concentrations (80%-95%-100%) of three minutes each and two baths of xylene for another three minutes.

RESULTS

Histopathological examination: Liver sections from diethylnitrosamine control group showed disturbed parenchyma with loss of lobular pattern, appearance of malignant hepatocytes of moderate to marked nuclear anaplasia. In addition, intralobular inflammatory cell infiltration, collagen deposition and congested hepatic veins were evident. On the other hand, tissue sections from mice treated with sorafenib or itraconazole as a

monotherapeutic agent or as adjuvant with sorafenib showed marked regression of malignant changes (Fig. 1) according to grading system described by Bosman, Carneiro [18].

DISCUSSION

The present study investigates the effect of itraconazole on diethylnitrosamine -induced hepatocellular carcinoma in mice. We provide an evidence based on histopathological findings that itraconazole alleviates experimental hepatocellular carcinoma. Malignant enlarged hepatocytes revealed after histological analysis of diethylnitrosamine-treated mice along with anaplasia are both the evidence of hepatocellular carcinoma in our model. We demonstrated marked improvement in liver histology after itraconazole treatment which was more significant after the combination treatment. Our results are coincident with Kadasa, Abdallah [19].

Based on histological examination, a significant impact to the anti-cancer effect of sorafenib was added by itraconazole at the present schedule of dosing and conditions. However, further studies based on biochemical and molecular analysis are required to investigate the potential synergistic activity of itraconazole as co-adjuvant to other chemotherapies such as sorafenib. Finally, our study provided a histological evidence that itraconazole may act as an anti-tumor agent and may add to the anti-tumor activity of other chemotherapies such as sorafenib. In addition, itraconazole is a promising candidate for further experimental and clinical studies in the treatment of hepatocellular carcinoma.

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

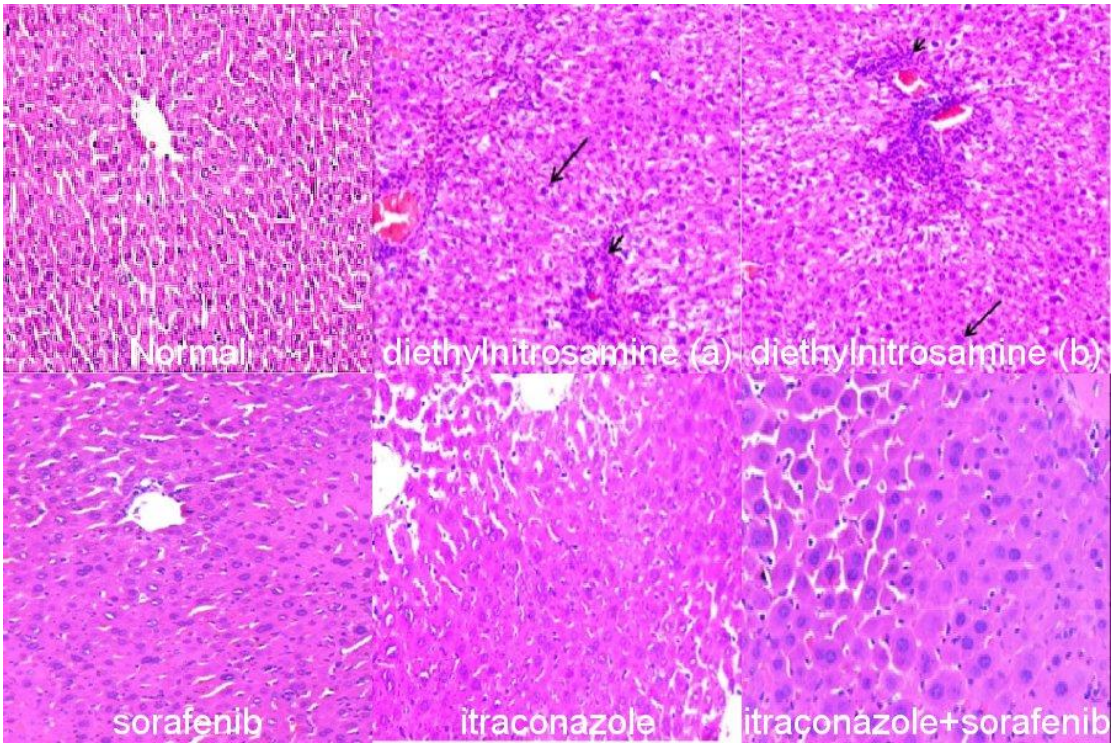

Fig.1 Representative Histology of liver tissue sections from normal control group, diethylnitrosamine control group, sorafenib treated group, itraconazole treated group, itraconazole+sorafenib treated group (Hx&E X200).

REFERENCES

1. El Serag, H.B. and F. Kanwal, Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? Hepatology, 2014. **60**(5): p. 1767-1775.
2. Chen, C.J., et al., Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA, 2006. **295**(1): p. 65-73.
3. Di Marco, V., et al., Sorafenib: from literature to clinical practice. Ann Oncol, 2013. **24 Suppl 2**: p. ii30-7.

4. Brito, A.F., et al., Targeting Hepatocellular Carcinoma: What did we Discover so Far? Oncol Rev, 2016. **10**(2): p. 302.
5. Saber, S., Angiotensin II: a key mediator in the development of liver fibrosis and cancer. Bulletin of the National Research Centre, 2018. **42**(1): p. 18.
6. Saber, S., et al., Lisinopril inhibits nuclear transcription factor kappa B and augments sensitivity to silymarin in experimental liver fibrosis. International Immunopharmacology, 2018. **64**: p. 340-349.
7. Saber, S., et al., Liver Protective Effects of Renin-Angiotensin System Inhibition Have No Survival Benefits in Hepatocellular Carcinoma Induced By Repetitive Administration of Diethylnitrosamine in Mice. Open Access Macedonian Journal of Medical Sciences, 2018. **6**(6): p. 955-960.
8. Saber, S., et al., Perindopril, Fosinopril and Losartan inhibited the progression of diethylnitrosamine-induced hepatocellular carcinoma in mice via the inactivation of nuclear transcription factor kappa-B. Toxicology letters, 2018.
9. Saber, S., et al., Losartan, an angiotensin-II type 1 receptor blocker, attenuates CCl4-induced liver fibrosis with a positive impact on survival in mice. World Journal of Pharmaceutical Sciences, 2017. **5**(12): p. 121-126.
10. Saber, S., et al., Renin–angiotensin system inhibition ameliorates CCl4-induced liver fibrosis in mice through the inactivation of nuclear transcription factor kappa B. Canadian journal of physiology and pharmacology, 2018(999): p. 1-8.
11. Abdel-Ghany, R., et al., Blockade of PGE2, PGD2 Receptors Confers Protection against Prepatent Schistosomiasis Mansoni in Mice. Journal of the Egyptian Society of Parasitology, 2015. **45**(3): p. 511-520.
12. Horton, J., Albendazole: a review of anthelmintic efficacy and safety in humans. Parasitology, 2000. **121**(S1): p. S113-S132.
13. Pourgholami, M., et al., In vitro and in vivo suppression of growth of hepatocellular carcinoma cells by albendazole. Cancer letters, 2001. **165**(1): p. 43-49.
14. Villanueva, A., et al., Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis, 2007. **27**(1): p. 55-76.
15. Pourgholami, M.H., et al., Potent inhibition of tumoral hypoxia-inducible factor 1α by albendazole. BMC cancer, 2010. **10**(1): p. 143.
16. Saber, S., et al., Perindopril, fosinopril and losartan inhibited the progression of diethylnitrosamine-induced hepatocellular carcinoma in mice via the inactivation of nuclear transcription factor kappa-B. Toxicology Letters, 2018. **295**: p. 32-40.
17. Kuczynski, E.A., et al., Effects of Sorafenib Dose on Acquired Reversible Resistance and Toxicity in Hepatocellular Carcinoma. Cancer Res, 2015. **75**(12): p. 2510-9.
18. Bosman, F.T., et al., WHO classification of tumours of the digestive system. 2010: World Health Organization.
19. Kadasa, N.M., et al., Hepatoprotective effects of curcumin against diethyl nitrosamine induced hepatotoxicity in albino rats. Asian Pac J Cancer Prev, 2015. **16**(1): p. 103-8.