Rescue control experiments to regain the knockdown anticancer effects of repurposed drugs on fibrosarcoma in hamsters

Kosta J. Popović, PhD in Pharmacy[1], Dušica J. Popović, MD, PhD[2], Dejan Miljković, MD, PhD[3], Dušan Lalošević, MD, PhD[3], Zana Dolićanin, MD, PhD[2], Ivan Čapo, MD, PhD[3] and Jovan K. Popović, MD, PhD[4]

[1]Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad; Serbia
[2]Department of Biomedical Sciences, State University of Novi Pazar, Novi Pazar, Serbia
[3]Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[4]Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

INTRODUCTION

Literature analysis shows that repurposed non-oncological drugs, such as metformin and disulfiram, act through a lot of same anticancer mechanisms, on the same targets, in the same direction. Also, for majority of these mechanisms, non-oncological drug deoxycholic acid acts in the opposite pro-cancer direction. If the rescuing target/protein is expressed at sufficient level that it can reciprocally modulate the pathway of interest, the rescue experiment provide evidence for underlying mechanism of investigated process.

To test whether NF-κB and HIF-1α inhibition underlies the anticancer mechanism of examined drug combination, metformin and disulfiram, we comedicated a NF-κB and HIF-1α stimulator deoxycholic acid in order to rescue NF-κB and HIF-1α activity and block the hypothesized anticancer mechanism of the examined repurposed drug combination. For better understanding how the metformin and disulfiram combination exhibits the hypothesized anticancer effect, we overexpressed NF-κB, HIF-1α and downstream targets (VEGF, P-gp, MRPs) by deoxycholic acid.

METHODS AND MATERIALS

The study was performed on Syrian golden hamsters (age, 10-12 weeks; weight, ~80 g). The hamsters were randomized into groups (n=6 hamsters/group). The subcutaneous inoculation of 1 ml of BHK-21/C13 cell suspension ($2x10^6$ cells/ml) was performed for development of a hamster fibrosarcoma tumors. During 18 days after tumor inoculation, animals with experimental fibrosarcoma were treated orally (via a metal gastric tube) with investigated drugs.

We compared anticancer effects and safety of metformin and disulfiram combination (doses equivalent to usual human doses by normalization to body surface area) in hamster fibrosarcoma with: a) control (with inoculated tumors, to test efficacy and toxicity); b) two drug combination (metformin and disulfiram); c) doubled doses of each drug from examined combination given alone (to enhance possible anticancer effects of each single drug); d) metformin and disulfiram combination with addition of deoxycholic acid (doses equivalent to human), in order to definitely confirm anticancer efficacy of the combination and to elucidate potential mechanism of anticancer action; e) deoxycholic acid alone.

The animals were sacrificed 19 days (tumors ~2-3 cm) post-inoculation.

The blood was collected for hematological and biochemical analyses.

After sacrifice, the tumors were excised, photographed and weighed, their diameters were exactly measured, and the exact tumor volume was determined using the standard water volume displacement method.

Tumor sections (4 μm) were evaluated pathohistologically for the verification of tumor growth, tissue penetration, expansion of necrosis and hemorrhagic areas. Immunohistochemical staining was used to determine: mutational status (p53); tumor proliferation (Ki-67, PCNA); neoangiogenesis (CD34, CD31); glucose metabolism (GLUT1); NO metabolism (iNOS) and apoptosis (COX4, Cytochrome C, caspase 3) in the tumor slices.

The vital animal organs (brain, heart, lungs, liver and kidneys) were analyzed histopathologically and toxicologically.

RESULTS

As metformin and disulfiram combination shows synergistic anticancer effects on hamster fibrosarcoma, that can be aborgated with deoxycholic acid, NF-κB and HIF-1α can be possible underlying cancer modulatory mechanism.

There were no notable toxicity on main organs.

Oral administration of the drug to the hamster via gastric gavage

CONCLUSIONS

The obtained results indicate that the metformin and disulfiram combination can be recommended for further investigations in oncology.

ACKNOWLEDGEMENTS

Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research, grants no. 003074336-2024-09418-003-000-000-001/1 (JM) and 142-451-2626/2021 (DL).

KEYWORDS

hamster fibrosarcoma; metformin; disulfiram; NF-κB; HIF-1α

CONTACT

Kosta J. Popović
Faculty of Medicine, University of Novi Sad
Email: kosta.popovic@mf.uns.ac.rs
Website: www.mf.uns.ac.rs