



Construction, analysis and correlation of dose response logistic curves for repurposed drugs anticancer effects on fibrosarcoma in hamsters



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INTRODUCTION

Logarithmic dose response curves are typically „S“-shaped, monotonic and can be fit to a classical Hill equation. The Hill equation is logistic curve – an extension of the sigmoid functions.

Pleiotropy in anticancer mechanisms and pleiotropic modes of action of some cheap non-oncological, non-toxic marketed pharmaceuticals and vaccines enable promising research approach for discovering and targeting anticancer mechanisms and design of new efficacious anticancer treatments with minimal toxicity and cost.

Although *in vitro* (cell lines) anticancer effects of some nononcological pleiotropic drugs, such as metformin and itraconazole are reported, *in vivo* (experimental animals) and clinical investigations, especially of their combinations, are not yet conducted.

METHODS AND MATERIALS

The study was conducted on Syrian golden hamsters (10-12 weeks old; weight, ~80 g). The animals were randomized into groups (6 hamsters per group). The subcutaneous inoculation of 1 ml of BHK-21/C13 cell suspension (2×10^6 cells/ml) was performed for development of a fibrosarcoma in hamsters. 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 examined repurposed drugs 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 itraconazole); c) doubled doses of each drug from examined combination given alone (to enhance possible anticancer effects of each single drug); d) dose response effectivity validation experiments (tumor dimensions vs. percent of human equivalent doses of each drug in combination: 0%, 50%, 100% and 200%, obtaining typical logarithmic S-shaped sigmoid curve - Hill logistic dose response curve characteristic for pharmacodynamic effect), in order to definitely confirm anticancer efficacy of the combination; furthermore, comparing results of c) and d) will enable to differ additive effect from potentiating synergism (Combination Index - CI analysis).

The animals were sacrificed 19 days (tumors ~2-3 cm) post inoculation. To sacrifice the animals, a dose of 90 mg/kg pentobarbital was administered intraperitoneally.

The volume (2-3 ml) of the total collected blood was used for blood hematological and biochemical analyses. Following exsanguination and animal death, vital organs (heart, lungs, liver, kidneys and brain) were removed.

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 slices (4 μ m) were assessed pathohistologically (for the verification of tumor growth, tissue penetration, expansion of necrosis and hemorrhagic areas, proliferation), and immunohistochemically to assess: mutational status (p53); tumor proliferation (Ki-67 and PCNA); neoangiogenesis (CD34 and CD31); glucose metabolism (GLUT1); NO metabolism (iNOS) and apoptosis (COX4, Cytochrome C and caspase 3) in the tumor specimens. The weight of the hamsters was recorded to evaluate possible side effects of examined drug combinations. The vital animal organs (brain, heart, lungs, liver and kidneys) were analyzed histopathologically, and toxicologically.

RESULTS

Validation of combinatory anticancer effects by dose response effect (Hill S-shaped curves and Combination Index – CI analysis) confirmed our earlier findings of synergistic metformin and itraconazole interaction *in vivo*. Metformin and itraconazole combination have $CI < 1$ for all investigated anticancer effects in the experiment.

KEYWORDS

hamster fibrosarcoma; metformin; itraconazole; synergism; dose response



Inoculation of Syrian golden hamsters with 1 ml of BHK21/C13 cell culture (2×10^6 cells/ml)

CONCLUSIONS

As metformin and itraconazole combination shows synergistic dose response antitumor effects on hamster fibrosarcoma without toxicity, with $CI < 1$ for all analysed tumor characteristics, the combination is recommended for further investigations in oncology.

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