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Original research article

Hepatocellular cancer cell lines, Hep-3B and Hep-G2 display the pleiotropic response to resveratrol and berberine

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ABSTRACT

Purpose: Human carcinoma cells with different p53 status exposed to a combination of bioactive substances, resveratrol and berberine, revealed different responses in cell viability via p53-dependant apoptosis pathway activation.

Materials and methods: Using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, we investigated various and opposing effects in hepatocellular carcinoma cells, Hep-G2 and Hep-3B with different p53-status.

Results: Cells decreased in viability after treatment with dose-dependent concentrations of resveratrol and berberine. Hep-3B p53 mutants were more sensitive in comparison to the p53 wild type Hep-G2 cell line. A synergistic effect was observed after treatment of Hep-3B cells with a combination of resveratrol/berberine ratios in favor of resveratrol (2:1, 3:1). The results suggest that an effective concentration of berberine, in the presence of resveratrol, could be decreased even to 50% (half the IC_{50} for berberine) in cancer treatment. Combined treatment with berberine and resveratrol, at the investigated concentrations and fractions, significantly reduces the viability of wild type p53 Hep-G2 and null p53-mutant Hep-3B cells by 20% and 40%, respectively.

Conclusions: Stronger toxic effects on viability and proliferation were observed in Hep-3B cells what is consistent with the assumptions that null p53-mutants activate apoptosis canonical pathway. In conclusion, p53 status in human hepatocellular cancer cell lines modulates responses to plant-derived therapies.

1. Introduction

In developed countries, one of the major health problems affecting populations is obesity. Obesity leads to an increased risk of developing other health disorders including colon and breast cancers [1,2]. Adipose tissue delivers regulators, which influence hepatocellular carcinoma (HCC), also called hepatoma [3], a high mortality liver cancer [4]. Hepatomas are mainly known for their associations with hepatitis B (HBV) and C (HCV) viral infections and chronic alcohol intake [5]. Current pharmacotherapy is mostly indicated for patients with HCC in advanced stage C of

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Barcelona Clinic Liver Cancer (BCLC) classification [6]. It consists of sorafenib or lenvatinib in the first-line and regorafenib and - less active – nivolumab in the second-line therapy. The addition of plant-derived curcumin during anticancer therapy of Hep-3B cells inhibited cell death via the apoptotic pathway [7]. The reports show, that in p53-null liver cancers the AKT/PTEN/FOXO4 pathway for apoptosis is a potential target for treatment [7]. Besides exogenous agents, the mechanism of the pathway up- or down-regulation via intracellular regulators, such as miRNAs, was studied. For osteogenic differentiation of MC3T3-E1 cells, the PI3K-Akt molecular pathway under miR-34c control was also examined. This molecular loop was also down-regulated by vaspin, an endogenous adipocytokine produced by adipose tissues, which can inhibit bone reconstruction and regeneration *in vitro* [8,9]. Plant-derived molecules such as resveratrol from grapes or the alkaloid berberine from barberry have been present in the human diet since the time of ancient civilizations, just as coffee is now known to contain a set of bioactive compounds what has been reported in studies on the development and progression of colorectal cancer [10]. Resveratrol and berberine, plant-derived molecules, are known to have anti-cancer properties. In our previous studies and in other authors' report [11–13], berberine has been found to act as an anti-proliferative and pro-apoptotic agent on squamous carcinoma SCC-25 cell line. The effect of berberine improves significantly when combined with silver nanoparticles, where the reported IC_{50} was reduced to 50% (from 25 μg/mL to 12.5 μg/mL) in the presence of AgNPs [11]. These natural molecules, as well as vaspin, have already been investigated on human SCC-25 cells and human Hep-3B cells and were found to have different cellular effects [11, 13]. The antiproliferative effects were mostly caused by an additive influence of resveratrol and berberine that effectively arrested the cell cycle and induced cell death. Mutation of tumor suppressor gene TP53 is the most common and well-studied cancer gene. The p53 protein participates in cell cycle arrest, DNA repair, and controls the mechanisms of apoptosis [14,15]. For *in vitro* studies, cellular models for liver cancers with different p53 statuses were used [16–18]. In this manuscript we present studies that focus on the resveratrol and berberine impact on the Hep-G2 (p53 wild type) and Hep-3B (p53 null mutant) cell lines, our aim was to investigate the pro-apoptotic potential upon p53 regulated pathways.

2. Materials and methods

2.1. Cell culture

HCC (Hep-3B, p53-negative mutant; and Hep-G2, p53-wild type) cells from ATTC (Manassas, VA, USA) were a kind gift from Dr. Marek Rusin from Maria Sklodowska-Curie Memorial Cancer Center, National Institute of Oncology's collection (Gliwice, Poland). Cells were grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12; PAN-Biotech GmbH, Aidenbach, Germany) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; EURx, Gdansk, Poland) and 100 units/ml penicillin and 100 μg/ml streptomycin (Merck KGaA, Darmstadt, Germany) at 37 °C in a humidified atmosphere with 5% CO_2. For 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, cells were seeded in 96-well plates at 1×10^4 cells/well in 0.2 ml medium 24 h before drug treatment.

2.2. Plant-derived berberine and resveratrol

Concentrated stock solutions (1 mg/ml) of berberine chloride (Merck KGaA, Darmstadt, Germany) and resveratrol (Merck KGaA, Darmstadt, Germany) were prepared in sterile H_2O (Merck KGaA, Darmstadt, Germany) and absolute ethanol (POCH, Gliwice, Poland), and were stored in −20 °C. For experiments, desired concentrations of one or combined compounds were prepared directly in the growth medium. The control medium was supplemented with ethanol if used as background control. Experimental concentrations of working solutions were as follows: 0.078, 0.15, 0.31, 0.68, 1.25, 2.5, 5 and 10 μg/ml, as described previously [13].

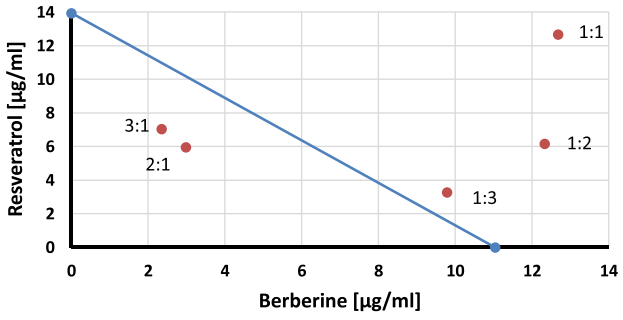
2.3. Cell viability assay

Cells were incubated for 24 h with the compounds, and then washed three times with phosphate buffered saline (PBS; PAN-Biotech GmbH, Aidenbach, Germany). They were then incubated for 2 h with 20 μl of MTS solution (Promega GmbH, Walldorf, Germany) in 100 μl of PBS (PAN-Biotech GmbH, Aidenbach, Germany) until the color in the control changed from light yellow to brown and the colorimetric reaction developed. The levels of absorbance were measured at a wavelength of $\lambda = 490$ nm by microplate spectrophotometer (Epoch; BioTek Instruments, Inc., Winooski, VT, USA) and expressed as a fold change compared to the untreated control. A dose block matrix for isobologram calculations, followed by viability MTS assay (Promega GmbH, Walldorf, Germany) was prepared according to described methods [13,19,20]. On the 96-well plate, dose matrix blocks were constructed by dispensing berberine at the concentrations of 10, 5, 2.5, 1.25, 0.65, 0.31, 0.15 and 0 μg/ml; the same doses were used for resveratrol, and for a mix of both compounds. Across the 8 × 8 block of rows and columns, a matrix for every combination of doses for both compounds was obtained. After 24 h of incubation, the results from MTS assay (Promega GmbH, Walldorf, Germany) allowed for a "viability heatmap" presentation (Fig. 1). Characterization of antagonism, synergy, and/or additivity was calculated from block matrices, as described previously [13].

2.4. Microarray analysis

In order to determine changes in signaling pathways after berberine dosage in Hep-G2 cells, we used a dataset from Lo et al. [21] access number E-GEOD-47822. The cut-off point was 10% of change between control and berberine treated cells. Next, sets for up- and down-regulated

A



B

IC_{50}	Resveratrol	Berberine
	13.94	11.04
resveratrol:berberine ratio	experimental data [μg/ml]	
01:01	12.68	12.68
01:02	6.17	12.33
02:01	5.96	2.98
03:01	7.04	2.35
01:03	3.26	9.79

Fig. 1. Isobologram for combined berberine + resveratrol treatment (A) and IC_{50} (B) calculated from doses matrix block, followed by 24 h MTS assay for Hep-3B cells.

genes were analyzed with ConsensusPathDB [22] to find over-represented pathways or gene ontology (Supplementary File S1).

2.5. Statistical analyses

The results are expressed as means ± standard deviation (SD) from three independent experiments. Isobologram was prepared as described in Skonieczna et al. [13] and Tallarida [19,20]. Results were analyzed in MS Excel 2010.

2.5.1. Isobologram

Numerical characterizations of antagonism, synergy, and/or additivity were calculated as previously described [13]. The results are presented as an isobologram chart for IC_{50}, calculated from MTS assay for berberine and resveratrol alone, in a few different ratio combinations of both compounds: 1:1, 1:2, 2:1, 3:1, and 1:3 (Fig. 1). The data are presented as mean values for substance concentrations for IC_{50} doses.

2.6. Ethical issues

Ethical approval was not required for this study. This study is in accordance with the 1964 Declaration of Helsinki and its later amendments.

3. Results

The dose-dependent effects on cells *in vitro* for novel molecule applications are rather complex and non-specific. Responses from live-cell assays and observations are also interpretative and pleiotropic. Multiparameter assays, chosen for pharmacokinetic interactions of berberine and resveratrol on HCCs, deliver a lot of information about molecules' synergy, additivity, and/or antagonism on Hep-3B and Hep-G2 cell lines. From one MTS cytotoxicity assay, within 24 h of incubation with resveratrol and berberine alone or a combination of both at different concentrations, we have created an isobologram for plant-derived molecules on cell application for anticancer treatments (statistical significance is followed by *t*-test calculation; Table 1). Firstly, a "viability heatmap" showed a linear dose-dependent response for Hep-3B cells when resveratrol or berberine was tested alone (Table 2, top row and left column on the heatmap). The cells started to die when the concentration of berberine or resveratrol increased from 0 up to 10 μg/ml, similar for both compounds. Despite the linear response, the combination of both tested molecules began to complicate cellular responses (Table 2, rows and columns in the middle).

Calculations of effective doses from isobolograms allowed for more precise interpretations of which concentrations of berberine and/or resveratrol should be used to reduce side-effects when anticancer activity increases. Calculated from MTS assay concentrations, showed reduction of the viability of Hep-3B cells by 50%, the IC_{50} for berberine was 11.04 μg/ml and for resveratrol 13.94 μg/ml (Fig. 1).

Combinations of berberine and resveratrol at different ratios enhanced toxic effects on Hep-3B cells. Ratios in favor of berberine (1:2, 1:3) and equal (1:1) act antagonistically (Fig. 1. A, orange dots above the blue trend line), for the same result (IC_{50} for a single drug) the highest doses of drugs need to be taken than those calculated from the theoretical additive line (blue line in Fig. 1. A). The highest antagonistic effect was observed with an equal ratio of resveratrol to berberine (1:1, Fig. 1. A). In the situation when berberine was lowered, that is added in ratios (resveratrol:berberine) 3:1 or 2:1 (Fig. 1. A, orange dots below the blue trend line), synergistic effects were observed. The blue trend line represents additive effect, calculated from the experimental IC_{50} data for both compounds. Experimental results allow for the selection of appropriate combinations of berberine and resveratrol in order to obtain effective cytotoxic effects on Hep-3B cells, by lowering initial concentrations of compounds (Fig. 1. B). Taking this information and additional 24-h MTS assays into account, a fixed value for berberine or resveratrol could be determined. For example, using low doses (0.68 μg/ml) of resveratrol with variable spectrum doses of berberine (10, 5, 2.5, 1.25, 0.65, 0.31, 0.15 and 0 μg/ml), there was a 20% reduction in Hep-3B viability when the highest berberine dose (10 and 5 μg/ml) was used (Fig. 2. A). Opposite treatments, with constant doses of berberine (0.68 μg/ml) and variable resveratrol doses resulted in better reduction effects in Hep-3B viability where a 40% viability reduction was observed (Fig. 2. A). These good effects were still present even when the doses were reduced to 0.31 and 0.15 μg/ml, for berberine and resveratrol, respectively (Fig. 2. B and C). The effective doses for berberine or resveratrol could even be toxic at the dose of 1.25 μg/ml when the second compound in the combined treatment was added at a low dose of 0.15 μg/ml (Fig. 2. C). Such treatments did not reach IC_{50} values for berberine alone, however, the cytotoxic effects against healthy cells could be reduced, especially in anticancer treatments.

Concerning the p53 status in different cell lines of HCC, a 24-h MTS assay in Hep-G2 (p-53 wild type) and Hep-3B (p-53 mutant) was done. Based on isobologram calculations and experimental results, an IC_{50} for berberine equal to 11.04 μg/ml and for resveratrol equal to 13.94 μg/ml was determined (Fig. 1. B) at the mutant p53 Hep-3B cell line. Although, the highest used doses did not reach such concentrations (the highest dose established was at 10 μg/ml) a 50% viability reduction for Hep-G2 and Hep-3B was obtained (Fig. 3. A and B). Surprisingly, the p53 wild type Hep-G2 cell line did not respond to the berberine alone, even at high doses of 5–10 μg/ml (Fig. 2. A). A strong stimulatory effect of berberine was observed, with 160% of control viability and proliferation improvement (Fig. 3. A). Berberine, when applied on its own, seemed to be more protective than cytotoxic to both cell lines, however, in the mutant p53 Hep-3B cell line there was only a ~10% viability reduction, in comparison to the untreated controls (Fig. 3. B). P53 status in both HCC cell lines affects cellular responses to berberine and resveratrol, and the lack of functional p53 protein lowered the resistance to anticancer treatment.

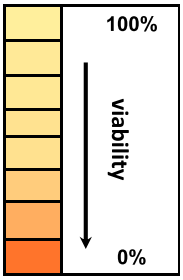
From the microarray analysis, we observed an overrepresentation of apoptotic process ontology in the upregulated group of genes – changed more than 10% of expression in HepG2 cells after 4 h treatment with 40 μM berberine [22]. Expression of several genes was elevated by more than 50%, including ANKRD1, EGR1, IER3 and others (Supplementary File S1).

Table 1
Statistical significance (p-value < 0.05) calculated from *t*-test indicated with bold text for Hep-G2 (A) and Hep-3B (B) cells viability following a 24 h MTS assay after resveratrol, berberine, and combined resveratrol + berberine treatments.

A	[μg/ml] $p < 0.05$	resveratrol	resv + berb	berberine
Hep-G2	10	4.920E-06	6.090E-09	3.213E-06
	5	4.253E-03	2.971E-01	1.799E-10
	2.5	8.424E-03	2.628E-04	3.125E-02
	1.25	3.290E-03	4.469E-03	3.082E-01
	0.6	1.501E-04	1.240E-04	1.927E-04
	0.3	5.026E-03	7.558E-01	3.506E-03
	0.15	7.086E-01	1.293E-01	4.628E-03
	0.078	2.021E-01	7.521E-01	3.524E-01
B	[μg/ml] $p < 0.05$	resveratrol	resv + berb	berberine
Hep-3B	10	7.630E-01	5.868E-03	3.071E-02
	5	1.773E-01	5.633E-03	8.471E-03
	2.5	2.933E-03	1.009E-03	1.316E-03
	1.25	7.094E-05	6.907E-05	6.496E-05
	0.6	1.877E-04	9.116E-04	1.163E-05
	0.3	5.565E-03	3.884E-03	7.002E-04
	0.15	1.075E-01	1.679E-02	7.093E-02
	0.078	2.504E-03	4.842E-03	8.832E-01

Table 2
Representative "viability heatmap" for Hep-3B cells 24 h after berberine, resveratrol, or combined berberine + resveratrol treatment; absorbance at 490 nm from dose matrix block followed by MTS assay (arbitrary units, a.u.).

The combination responses for berberine and resveratrol [µg/ml] in Hep-3B cancer cells, 24h MTS assay								
Resveratrol \ Berberine	0	0.15	0.31	0.68	1.25	2.5	5	10
0	100%	58%	54%	62%	60%	54%	51%	46%
0.15	103%	93%	93%	93%	84%	86%	74%	67%
0.31	106%	104%	95%	90%	99%	86%	77%	67%
0.68	91%	102%	97%	91%	95%	88%	70%	67%
1.25	104%	69%	75%	91%	70%	64%	65%	50%
2.5	87%	68%	58%	76%	69%	61%	58%	48%
5	51%	55%	50%	52%	53%	56%	48%	46%
10	52%	54%	51%	55%	52%	54%	48%	47%



4. Discussion

4.1. Resveratrol induced detoxification in hepatocytes

Resveratrol used on different cell lines resulted in different actions, ranging from reactive oxygen species (ROS) reduction [13] in squamous carcinoma SCC-25 to detoxification in a human hepatoblastoma cell line Hep-G2 [23]. It has been known for centuries that resveratrol has a protective function in the digestive system. It has been used as an antioxidant and antitoxic agent to support liver functions, especially in aiding the detoxification process [23]. The addition of resveratrol, at a concentration of 10 µM, to Hep-G2 cells for 24–48 h resulted in the activation of phase II enzymes used in toxic agent metabolism, i.e. two UDP-glucuronosyltransferases (UGT1A1 and UGT2B7) and one sulfotransferase isoform (ST1E1) [23]. Resveratrol derivatives were also well metabolized and, as a result, direct metabolites were observed, confirming the ability of Hep-G2 cells to support detoxification. Contrarily, resveratrol in sensitive cell lines, such as human U251, rat RG2, and C6 glioblastoma cell lines, showed toxic, rather than protective effects [24]. Resveratrol at a dose of 100 µM effectively arrested the cell cycle and induced apoptosis [24]. In combination with berberine, resveratrol induced oxidative stress in the SCC-25 cell line 24 h after administration [13]. A bi-modal action of resveratrol on cellular effects depended strongly on the used doses and the origin of model cell lines. Apoptosis pathways were activated after resveratrol administration due to the interaction of various survival and cell death signaling pathways [25]. Resveratrol could impact the cells by stimulating aggregation of death receptors (CD95 or TRAIL receptors) or by inhibiting Bcl-xL, survivin, Mcl-1, and Bcl-2 – apoptosis inhibitor proteins [25]. Contrastingly, resveratrol could also influence cell survival pathways, such as MAPK NF-κB and PI3K/Akt [25]. Hep-G2 cells, a p53 positive model cell line, responded with viability reduction after resveratrol administration (Fig. 3.), presumably with the induction of a p53-positive dependent apoptosis pathway [26].

4.2. Effects of berberine in hepatocytes

Berberine affects many functions of liver-related metabolic syndromes, including non-alcoholic fatty liver disease (NAFLD), obesity and type 2 diabetes [27]. During treatments administered by digestive route, good bioavailability was shown by berberine metabolites in liver tissues [28]. Multidirectional applications, apart from anti-cancer, were investigated on model cell lines of HCCs. Different berberine-based drugs influenced fat metabolism and oxygenation, cholesterol production, glucose metabolism, and TNFα/IL6 or insulin receptors sensitivities on hepatocytes [27]. In model Hep-G2 model cell lines subjected to 24 h of inflammatory stimulation, the production of pro-inflammatory factors, TNFα and IL6, was effectively reduced following berberine administration in a dose-dependent manner (0.1–10 µM) [29]. In insulin-resistant hepatocytes such activation has been associated with metabolic syndromes - type 2 diabetes and obesity, which have been epidemically reported as global problems in humans. The cellular effects of 72-h combination therapy, based on berberine (0.1–100 µM) with S-allyl--cysteine (SAC), affected the canonical apoptosis pathway via cytochrome *c* release and caspase-dependent cascade activation of caspase-3 and caspase-9. This finally reduced Hep-G2 cell viability and induced G1-phase cell cycle arrest [30,31]. In the present study, we observed that lower concentrations of berberine at doses of 5–10 µg/ml, compared to the literature, resulted in stimulated cell proliferation effects (Fig. 3). Only during combined treatments of berberine with resveratrol at low doses, we detected a lethal effect on both cell lines, Hep-G2 and Hep-3B (Table 2; Figs. 2 and 3). A dose-dependent action on cells could be modulated because of hormetic effects, lower doses of tested berberine were found to be more effective than higher doses (Fig. 2) [32].

4.3. p53 status in human HCC cell lines regulates responses to resveratrol and berberine

The p53 protein was first classified as an oncogene in 1979 by several groups [33,34]. In 50% of human cancer cases, the TP53 is found to be mutated [35,36]. After some time, p53 was discovered to be more of a "genome guardian" than an oncogene factor [37]. Its mutation at different stages of tumor progression may affect tumor malignancy and prognosis [38]. Hep-3B and Hep-G2 cell lines have different p53 statuses, Hep-G2 presented wild type p53, whereas Hep-3B presented deficient p53 [39]. It was shown that resveratrol could affect p53 proteins, prevent aggregation of mutated p53, and reduce cell migration and proliferation [40]. Qiu et al. [17] in their review stated that the p53 status in liver cancer model cells, Hep-3B and Hep-G2, has little to no impact on a cell's response. However, Schuster et al. [41] examined the response of hepatocytes upon resveratrol treatment and found that the activation of apoptosis, specifically in cleaved isoforms at caspase-3 level, was different at lower doses of resveratrol. In cells with wild type p53, cleavage of caspase-3 occurred at the highest dose (100 µM), but in the p53-deficient cells, the cleaved caspase-3 was present at every dose [41]. In our present study, the viability of Hep-3B after resveratrol treatment

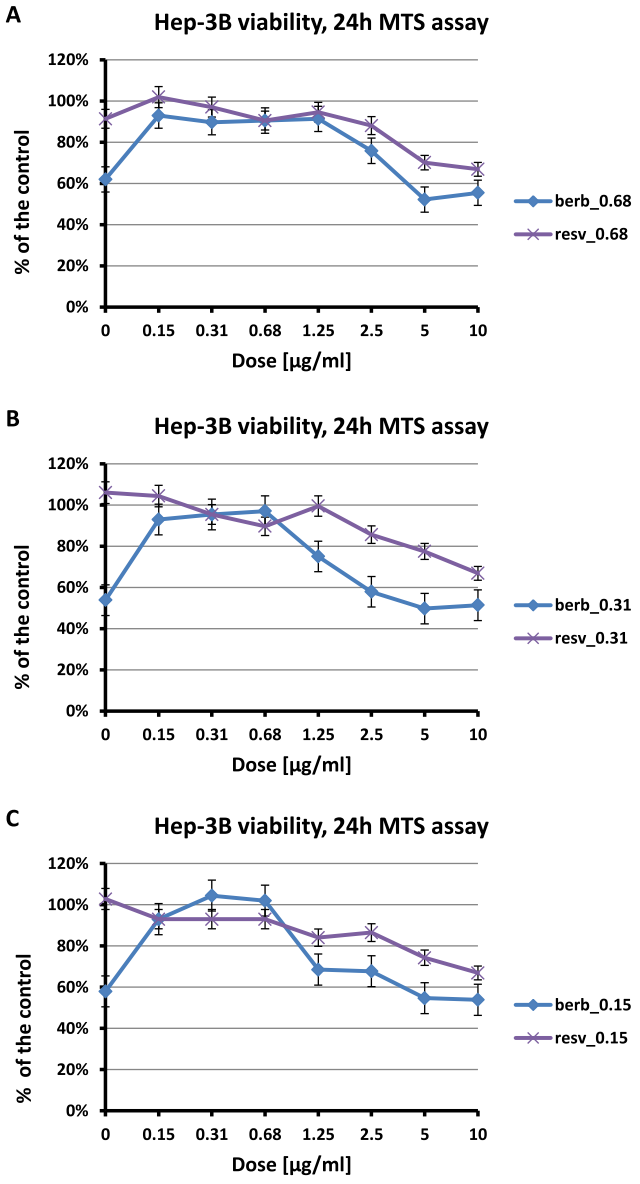


Fig. 2. Combined berberine + resveratrol treatment, followed by a 24 h MTS assay in Hep-3B cells. Fixed doses of berberine or resveratrol (A) 0.68 µg/ml, (B) 0.31 µg/ml and (C) 0.15 µg/ml with variable spectral doses of the other compound (10; 5; 2.5; 1.25; 0.65, 0.31; 0.15; 0 µg/ml). Data are presented as mean ± SD. Statistical significance is provided in bolded values in Table 1.

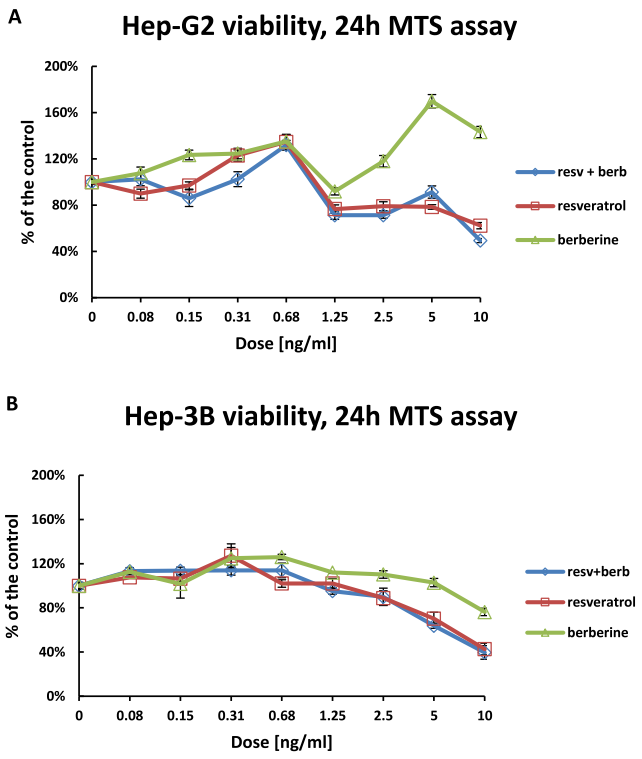


Fig. 3. Hep-G2 (A) and Hep-3B (B) cells viability following a 24 h MTS assay after berberine, resveratrol, or combined berberine + resveratrol treatments. Data are presented as mean ± SD. Statistical significance is provided in bolded values in Table 1.

was reduced more than Hep-G2 at the highest resveratrol dose tested, where 10 µg/ml is ~44 µM (Fig. 3.). Effective apoptosis induction was achieved with only 50% of the concentration used by Schuster et al. [41]. From calculated isobolograms (Fig. 1.), we determined effective doses of IC_{50} for berberine and resveratrol, with $IC_{50} = 11.04$ µg/ml for berberine and $IC_{50} = 13.94$ µg/ml for resveratrol. Both applications on the mutant p53 Hep-3B cell line were reduced from the previous squamous carcinoma SCC-25 cell line, where the doses of IC_{50} for berberine was 23 µg/ml and for resveratrol 9 µg/ml [11,13]. Surprisingly, resveratrol calculations for Hep-3B needed higher concentrations in combined treatments, whereas berberine was significantly reduced (Table 1; Fig. 1.). It is also promising, that the doses of both tested compounds could be reduced to low quantities, comparable to the doses presented to induce toxic effects on cancer in the Hep-3B cell line (Fig. 3.). Reported concentrations in the serum of model rats *in vivo* showed that berberine's low acute toxicity at lethal doses (LD_{50}) was 205 mg/kg, when administered intraperitoneally [28,42]. Therefore, lower concentrations of serum-detectable berberine could also be effective in anticancer strategies, even as a supplementary diet treatment for cancer patients. Although only 1% of berberine was reported as bioavailable in rats orally administered berberine solutions (200 mg/kg) dissolved in saline with a concentration of 40 mg/ml, phase I metabolites were found in different tissues. Up to 25 ng/ml of berberine metabolites have been reported in serum [42]. In a glass of red wine (250 ml), the average concentration of *trans*-resveratrol is 5 mg/L (or 20 mg/kg), this is 1000 times higher than what is considered a safe dosage [43]. Our experiments reported that lowered concentrations (Figs. 2 and 3.), appeared to be safer, and combined treatments with berberine resulted in a much more effective supplementary diet for anticancer treatments. Much of the United States population suffers from chronic obesity, this puts them at an increased risk of developing cancer since one-third of cancers are classified as obesity-related metabolic dysfunctions or chronic inflammations [44]. Fortunately, adipose tissue development could be residuary of adipose-derived stem cells (ADSCs), therefore, it may be a promising candidate for autologous cell-based regeneration therapies [45]. The existence of a feedback loop, involving nuclear factor κB (NF-κB), Lin28/Let7, and tumor necrosis factor α (TNF-α), has been described as necessary for ADSCs transformation [45]. Molecular mechanisms of cell death after vaspin treatment were studied in Hep-3B and in colorectal cancer cell lines, Caco-2 and HCT 116 [46,47]. Surprisingly, the adipocytokine-induced death was regulated on the ROS- and nitric oxide (NO)-dependent pathways with significant necrosis over apoptosis domination [47]. The main mode of action and the most desirable is a pro-apoptotic action. On the metabolic background of HCC, there are correlations with obesity, metabolic syndrome with insulin resistance, and NAFLD [48,49]. There are many other well-documented anticancer

strategies, most of which are based on natural molecules or artificial agent synthesis and combined therapies, also with standard e.g. radiotherapy for better cancer cells treatment [50].

5. Conclusions

Viability reduction of human HCC Hep-3B and Hep-G2 cell lines can be successfully enhanced by exposure to a mixture of bioactive phytochemicals - berberine and resveratrol. Pleiotropic response to these natural substances in the form of apoptotic hepatoma cell death is associated with p53-status, elucidating, that p53-dependant viability attenuation for Hep-3B p53-mutants is more apparent and significant compared to a wild type p53 Hep-G2.

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Data availability statement

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

Declaration of competing interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

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