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Joseph Alexis Zoa, Rahi-metou Njemguie Linjouom, Martin Nyangono Ndongo, Agathe Lafleur Ebongo, Rose Ngono Mballa

Corresponding author: Joseph Alexis Zoa, Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Ebolowa, Sangmelima 599, Cameroon. 1918093836@qq.com

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Determination of the half-maximal inhibitory concentration (IC50) of the Quercetin-Hyaluronic Acid Complex on the HepG2 cell line of hepatocellular carcinoma

Joseph Alexis Zoa[1,2,&], Rahi-metou Njemguie Linjouom[1], Martin Nyangono Ndongo[1,3], Agathe Lafleur Ebongo[4], Rose Ngono Mballa[5]

[1]Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Ebolowa, Sangmelima 599, Cameroon, [2]Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Diseases, Gannan Medical University, Ganzhou 34100, China, [3]Department of Galenics and Pharmaceutical Legislation, Faculty of Medicine

and Biomedical Sciences, The University of Yaoundé I, 366 Yaoundé, Cameroon, [4]Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, The University of Yaoundé I, Yaoundé 3316, Cameroon, [5]Department of Pharmacology and Traditional Medicine, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, 366 Yaoundé, Cameroon

[&]Corresponding author

Joseph Alexis Zoa, Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Ebolowa, Sangmelima 599, Cameroon

Abstract

Introduction: *Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer worldwide. Certain HCC cell lines overexpress the CD4 receptor, making it a preferential target for the quercetin-hyaluronic acid complex [QU-HA]. This study aims to evaluate the antiproliferative effect of [QU-HA] on HepG2 cells and determine its half-maximal inhibitory concentration (IC50) on these cells.* ***Methods:*** *Cell viability was measured using a WST-8 cell counting kit, following the manufacturer's instructions. The IC50 was calculated by linear regression, correlating the percentage of cell survival with the concentration of [QU-HA] (expressed in µM). Data were analyzed using GraphPad Prism software.* ***Results:*** *[QU-HA], at concentrations of [22.72; 18.18; 9.09; 4.54; 2.72; 0.909; 0.087 and 0.043] µM, significantly inhibited the growth of HepG2 cells. A concentration-dependent reduction in cell viability was observed after 24 hours of treatment. The 24-hour IC50 for HepG2 cells was determined to be 16.45 µM.* ***Conclusion:*** *[QU-HA] is capable of inhibiting 50% of HepG2 cell proliferation in a concentration-dependent manner, with an effective dose of 16.45 µM. These results suggest that [QU-HA] could be a promising therapeutic agent for the treatment of hepatocellular carcinoma.*

Introduction

Cancer is the second leading cause of death worldwide, following cardiovascular diseases [1]. In 2022, Cameroon recorded 19564 new cancer cases and 12798 deaths. Among these, primary liver cancer ranks as the fourth most common cancer (73844 new cases) and the third deadliest (70315 deaths), surpassed only by breast and cervical cancers [2]. This makes it a significant public health concern. The two main histological types of primary liver cancer are hepatocellular carcinoma (HCC) and cholangiocarcinoma [3], with HCC being the most prevalent form globally [4]. A persistent challenge is the limited availability of effective treatments, contributing to the disease's high mortality rate. Thus, novel therapeutic strategies are urgently needed to improve treatment outcomes.

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a plant-derived flavonol belonging to the flavonoid polyphenol group. Naturally present in many foods and herbs such as apples, red grapes, onions, raspberries, honey, cherries, citrus fruits, and green leafy vegetables, QU is a regular component of a normal diet [5]. It exhibits antibacterial, antioxidant, and protein kinase inhibitory properties, making it a unique multi-target compound with potential anticancer effects. Studies have shown that QU, at varying concentrations, suppresses tumor growth in multiple HCC cell lines, including SMMC-7221, MHCC97-H, MHCC97-L, HepG2, Hep3B, HuH-7 and C3A [6].

Despite its benefits, flavonoids are poorly absorbed and QU has low and highly variable bioavailability in humans (0-50%), along with rapid elimination (half-life of 1-2 hours) after ingestion. To overcome this limitation, QU is often combined with hyaluronic acid (HA) to form [QU-HA]. In this study, we demonstrated the in vitro

antiproliferative effect of [QU-HA] on the HepG2 hepatocellular carcinoma cell line and determined its half-maximal inhibitory concentration (IC50).

Methods

Chemical reagents

Phosphate buffered saline (PBS) and [QU-HA] were obtained from the Ganzhou Cancer Precision Medical Engineering Center (China). [QU-HA] was dissolved in dimethyl sulfoxide (DMSO), then diluted in cell culture medium to the indicated concentrations [22.72; 18.18; 9.09; 4.54; 2.72; 0.909; 0.087 and 0.043] µM. The final concentration of DMSO in the culture medium will not exceed 0.1% (v/v).

Cell lines, media and culture conditions

Hepatocellular carcinoma (HepG2) cell lines obtained from the American Type Culture Collection (ATCC) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium. The medium was supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin G sodium, 100 µg/mL streptomycin sulfate and 0.25 µg/mL amphotericin B) in monolayer in a humidified incubator at 37°C with 5% CO2 and 95% air and will be used in the logarithmic phase of growth. Media components were purchased from Solarbio.

In vitro cell viability

Cell viability was analyzed using a WST-8 cell counting kit (Beyotime, Jiangsu, China) as described [7]. Briefly, hepatocellular carcinoma cells were grown on 96-well plates to approximately 80% confluence, cells were starved for 24h in culture medium containing 0.5% SBF. Cells were then treated with [QU-HA] at the concentrations indicated, or with vehicle (0.1% DMSO) in quintuplicate (5-fold) for 24 hours. Cell viability was measured using CCK-8 according to the manufacturer's instructions.

Determination of inhibitory concentration 50 (IC50)

The IC50 was calculated by linear regression of the percentage survival as a function of drug concentration according to the formula [8]:

$$\text{Cell survival rate } (\%) = \frac{(A-C)}{(B-C)} \text{ x } 100$$

Where A = absorbance at each concentration of [QU-HA], B = absorbance at 0 µM [QU-HA], and C = absorbance of the blank

Statistical analysis

Data were analyzed in stages using the unpaired Student's t-test, which assumes that differences between groups are different. All bar charts represent the mean ± SEM (standard deviation of the mean) of at least three independent experiments. A P-value of less than 0.05 was considered statistically significant. IC50 was calculated using Graphpad Prism data analysis software version 8.0.2 (263).

Results

[QU-HA] inhibits hepatocellular carcinoma proliferation in vitro

Hepatocellular carcinoma (HepG2) cell lines cultured in vitro were exposed to varying concentrations of [QU-HA], and cell proliferation rates were measured using the CCK-8 assay after 24 hours (Table 1). [QU-HA] at concentrations of [22.72; 18.18; 9.09; 4.54; 2.72; 0.909; 0.087 and 0.043] µM significantly suppressed HepG2 cell growth. The reduction in HepG2 cell proliferation increased in a dose-dependent manner (Figure 1). After 24 hours of treatment, [QU-HA] inhibited HepG2 cell viability in a concentration-dependent fashion.

Real-time measurement of [QU-HA]'s IC50 in HepG2 cells

The half-maximal inhibitory concentration (IC50) of [QU-HA] in HepG2 cells was determined using GraphPad Prism data analysis software. Relative cell survival rates were plotted 24 hours after [QU-HA] treatment. As shown in Figure 2, the 24-hour IC50 value for HepG2 cells was calculated to be 16.45 µM.

Discussion

Our study demonstrates that the quercetin-hyaluronic acid complex [QU-HA] significantly inhibits proliferation of HepG2 hepatocellular carcinoma cells in a concentration-dependent manner, with an IC50 of 16.45 µM. Notably, this antiproliferative effect was achieved at remarkably low concentrations (as low as 0.043 µM), suggesting potent biological activity. These findings align with previous reports showing enhanced cytotoxicity of [QU-HA] compared to free quercetin in CD44-overexpressing cancer models [9,10]. The major strength of this work lies in its systematic evaluation of [QU-HA]'s dose-response relationship in a well-established HCC cell line. However, certain limitations must be acknowledged such as the study was conducted solely in vitro, requiring validation in more complex models, the precise molecular mechanisms underlying the observed effects remain to be elucidated, potential off-target effects were not assessed.

Our results corroborate and extend previous findings: Qi *et al.* [11] similarly reported superior efficacy of [QU-HA] versus quercetin alone in HepG2 cells. The observed IC50 (16.45 µM) closely matches Rianti *et al's.* report (15.899 µg/mL) [12], despite differing study endpoints. Notably, Wu *et al.* [13] found free quercetin required 95 µM for comparable effects in LM3 cells - a 5.8-fold higher concentration than our [QU-HA] complex. The enhanced potency of [QU-HA] likely stems from: HA-mediated targeting of CD44 receptors overexpressed in HCC [11,14]. Improved cellular uptake via receptor-mediated endocytosis. Potential stabilization of quercetin's bioactive conformation.

These findings have important translational implications: [QU-HA] represents a promising candidate for HCC therapy, particularly given CD44's frequent overexpression in liver cancers. Further research should: validate effects in animal models and patient-derived samples, investigate combination strategies with standard therapies, optimize formulation for clinical delivery, pharmacokinetic studies are needed to assess in vivo behavior, the 4-fold greater cytotoxicity of [QU-HA] versus free quercetin [9,10] underscores the potential of receptor-targeted delivery approaches to overcome quercetin's pharmacokinetic limitations while enhancing therapeutic efficacy against HCC.

Conclusion

This study demonstrated that [QU-HA] inhibited 50% of HepG2 hepatocellular carcinoma cell proliferation in a concentration-dependent manner at a dose of 16.45 µM. [QU-HA] could be a promising therapeutic agent in the treatment of hepatocellular carcinoma.

What is known about this topic

- *HCC is the most common primary liver cancer and remains a major global health challenge with limited treatment options;*
- *CD44 receptor overexpression in certain HCC cell lines (like HepG2) makes them a potential target for therapies using hyaluronic acid (HA)-based drug delivery;*
- *Quercetin, a natural flavonoid, has known anticancer properties, but its bioavailability and targeting efficiency can be improved through complexation with HA.*

What this study adds

- *Demonstration of [QU-HA]'s dose-dependent antiproliferative effect on HepG2 cells, with a calculated IC50 (16.45 µM), providing a quantitative benchmark for future studies;*
- *Evidence that low micromolar concentrations of [QU-HA] (as low as 0.043 µM) can inhibit HCC cell growth, suggesting high potency;*
- *Support for the potential of HA-mediated drug targeting in HCC therapy, leveraging CD44 receptor overexpression.*

Competing interests

The authors declare no competing interests.

Authors' contributions

All the authors have contributed to this work and approved the final version.

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Table and figures

Table 1: proliferation rate of HepG2 cells after treatment with [QU-HA] for 24h

Figure 1: effect of QU-HA on cell proliferation, the CCK-8 assay was performed to demonstrate the effect of [QU-HA] on HepG2 cells, viability was calculated after 24h. Statistical analysis is based on the mean ± SE of at least three independent tests, xP < 0.05, xxxP < 0.001 vs control

Figure 2: real-time measurement of [QU-HA] IC50 in HepG2 cells

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Table 1: proliferation rate of HepG2 cells after treatment with [QU-HA] for 24h

[QU-HA] (µM)	0	0.043	0.087	0.909	2.72	4.54	9.09	18.18	22.72
Proliferation (%)	99,07264	105,381	98,51258	90,01611	95,0697	80,13468	62,93334	51,50631	36,82744
	98,24774	94,35769	100,5401	99,967	89,68615	77,17372	66,43266	53,25162	44,60754
	98,4735	89,80337	94,48793	85,28813	75,37196	77,91179	61,30525	56,13443	46,73926
	101,3303	86,95095	87,28091	79,58764	77,47763	68,69896	55,88696	46,8999	55,86525
	102,8759	95,40401	81,91906	89,24765	86,49943	77,20411	67,37912	55,99116	41,37741
Moyenne	100	94,37939	92,54812	88,82131	84,82098	76,22465	62,78747	52,75668	45,08338
Ecart-type	2,018711	7,039288	7,809121	7,476294	8,287522	4,376952	4,588188	3,807276	7,090698

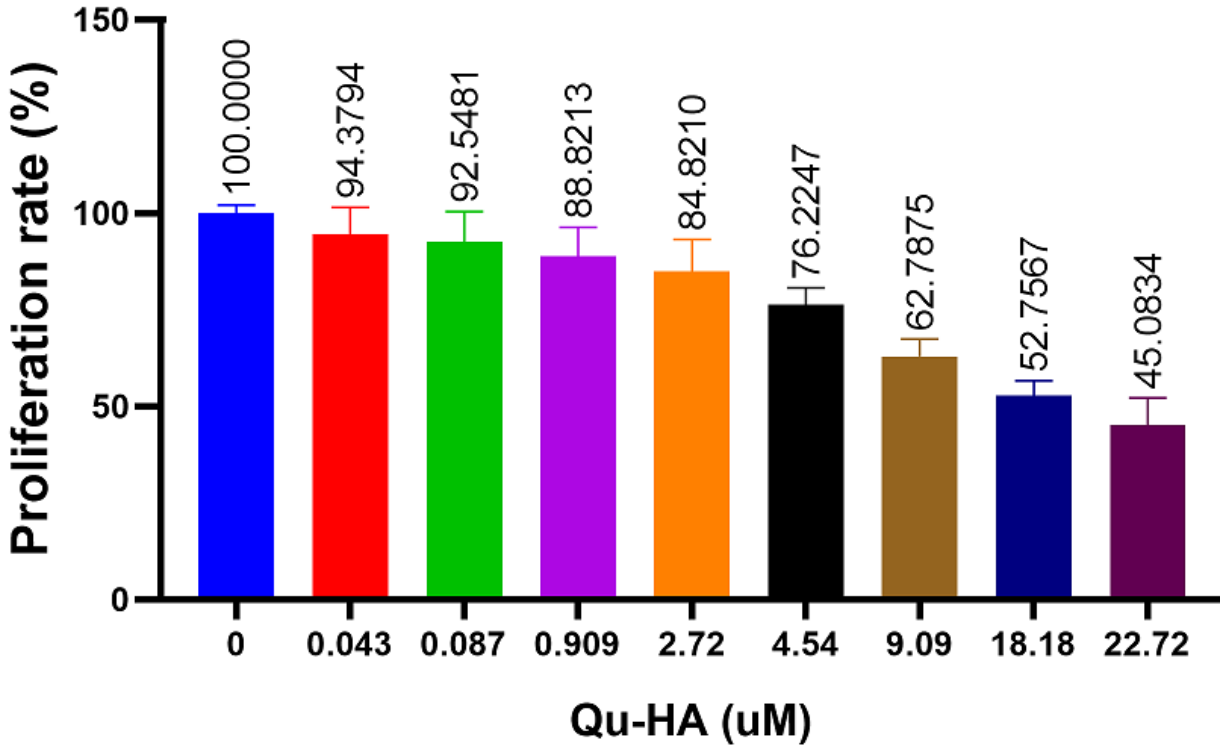

Figure 1: effect of QU-HA on cell proliferation, the CCK-8 assay was performed to demonstrate the effect of [QU-HA] on HepG2 cells, viability was calculated after 24h. Statistical analysis is based on the mean ± SE of at least three independent tests, xP < 0.05, xxxP < 0.001 vs control

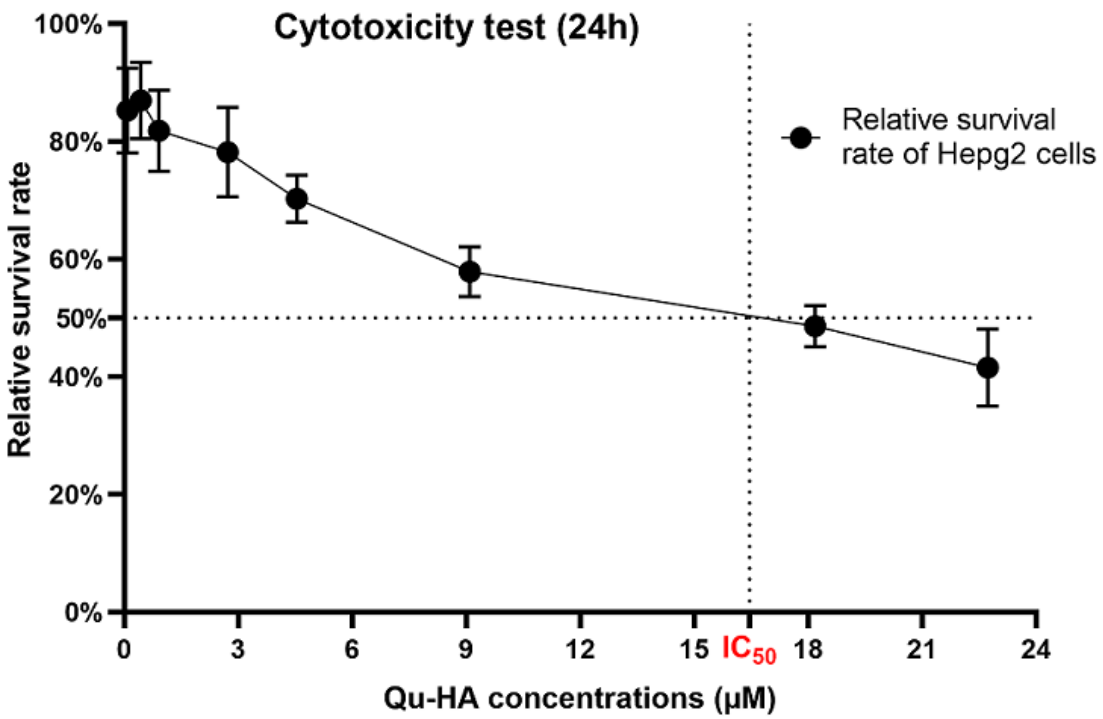

Figure 2: real-time measurement of [QU-HA] IC50 in HepG2 cells