ANTICANCER RESEARCH *40*: 6907-6914 (2020)
doi:10.21873/anticanres.14714

Flavopereirine Inhibits Hepatocellular Carcinoma Cell Growth by Inducing Cell-cycle Arrest, Apoptosis, and Autophagy-related Protein Expression

SAN-YUAN CHEN[1,2], CHUN-NUN CHAO[3,4], HSIN-YI HUANG[5] and CHIUNG-YAO FANG[5]

[1]*Department of Chinese Medicine, Ditmanson Medical Foundation Chiayi Christian Hospital, Chiayi, Taiwan, R.O.C.;*
[2]*Department of Sports Management, Chia Nan University of Pharmacy & Science, Tainan City, Taiwan, R.O.C.;*
[3]*Department of Paediatrics, Ditmanson Medical Foundation Chiayi Christian Hospital, Chiayi, Taiwan, R.O.C.;*
[4]*Department of Medical Laboratory Science and Biotechnology, Asia University, Taichung, Taiwan, R.O.C.;*
[5]*Department of Medical Research, Ditmanson Medical Foundation Chiayi Christian Hospital, Chiayi, Taiwan, R.O.C.*

Abstract. *Background/Aim: Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver. Resistance to chemotherapy and side-effects remain a challenge for treating advanced and recurrent HCC. Therefore, there is an emerging need to develop new drugs to treat HCC. Materials and Methods: We evaluated the anti-growth activity of flavopereirine in HepG2 and Huh7 HCC cell lines. Cell viability, cell-cycle profile, apoptosis, and autophagy-related protein expressions were analysed after flavopereirine treatment. Results: Flavopereirine treatment induced G_0/G_1 cell-cycle arrest, with an increase of sub-G_1 cells detected at the higher concentration and longer exposure to flavopereirine in HCC cells. Intrinsic and extrinsic pathways were involved in flavopereirine-induced apoptosis, as demonstrated by an increase of cleaved caspase 8 and 9 by western blotting. An alteration of autophagy-related protein expression was also found after flavopereirine treatment. Conclusion: Taken together, the current results indicate that flavopereirine exhibits good anticancer activity in HCC cells.*

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver (1). Although there has been considerable progress in the prevention, diagnosis, and treatment of HCC, the incidence and cancer-specific mortality continues to increase in many countries (2, 3). The risk factors of developing HCC include human hepatitis B virus and human hepatitis C virus chronic infection and cirrhosis, fatty liver disease and diabetes, alcoholic cirrhosis, as well as aflatoxin and aristolochic acid exposure (1, 4). At present, surgical resection is still the first-line treatment for patients with resectable liver cancer but around 70% of patients develop recurrent HCC (5, 6). Most patients diagnosed with HCC are at an advanced stage, hence, not suitable for surgical resection. Systemic chemotherapy and radiation are the preferred treatment for patients with advanced HCC, however, most drugs often have side-effects. Furthermore, resistance to drug treatment remains a challenge for chemotherapy in patients of advanced HCC (7, 8). Therefore, there is an emerging need to develop new drugs to treat HCC.

Flavopereirine is a β-carboline alkaloid which can be extracted from the bark of *Geissospermum* (Apocynaceae) (9, 10). It exhibits Leishmanicidal activity and selective DNA synthesis inhibition in cancer cells (11, 12). Furthermore, flavopereirine reduces the cell viability of drug resistance glioblastoma cells, but without an inhibitory effect on normal astrocytes (13). Recently, flavopereirine has been reported to suppress colorectal cancer and breast cancer cell growth (14-16), possibly through induction of cell-cycle arrest and apoptosis in these cancer cells. It seems that flavopereirine is a potential anticancer drug, but the anticancer activity of flavopereirine has not been assessed in HCC cells.

In the current study, we analysed the growth inhibition activity of flavopereirine in two HCC cell lines, HepG2 and Huh7 cells. The cell viability, cell cycle profile distribution, apoptosis and expression of autophagy-related proteins were evaluated after flavopereirine treatment in HCC cells.

Correspondence to: Dr. Chiung-Yao Fang, Department of Medical Research, Ditmanson Medical Foundation Chiayi Christian Hospital, 539, Chung Hsiao Rd., Chiayi 60002, Taiwan, R.O.C. Tel: +886 52765041 ext 5556, Fax: +886 52765041 ext 5555, e-mail: fcyo@ms72.hinet.net

Key Words: Flavopereirine, β-carboline alkaloid, hepatocarcinoma, apoptosis, autophagy, cell-cycle arrest.

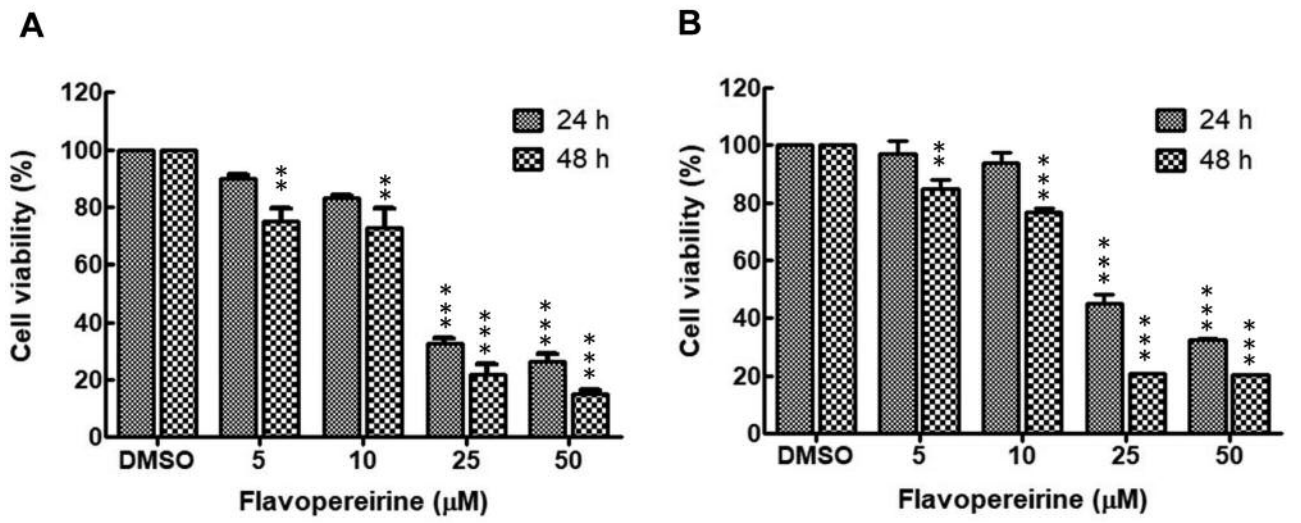

Figure 1. *Effects of flavopereirine on the growth of hepatocellular carcinoma (HCC) cells. HepG2 (A) and Huh7 (B) cells were treated with the indicated concentrations of flavopereirine for 24 or 48 h. Cell viability was determined by the CCK-8 assay and presented as the percentage of cell survival. Data are representative of at least three independent experiments and shown as mean±SD (*p<0.05; **p<0.01; ***p<0.001).*

Materials and Methods

Cell lines and cell culture. Human HCC cell lines HepG2 and Huh7 were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, NY, USA), containing 10% foetal bovine serum (FBS; Gibco) in a humidified atmosphere containing 5% CO_2 at 37˚C and tested for the absence of mycoplasma contamination.

Reagents and antibodies. Flavopereirine was purchased from ChromaDex, Inc. (Irvine, CA, USA). Proteins for western blotting analysis were extracted by M-PER mammalian protein extraction reagent buffer (Thermo Fisher Scientific, Waltham, MA, USA). The protease inhibitor was purchased from Millipore (Billerica, MA, USA). Primary antibodies for poly (ADP-ribose) polymerase (PARP), caspase 3, caspase 8, caspase 9, were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). The antibody against autophagy-related protein p62 (SQSTM1 monoclonal, mouse anti-human p62) was obtained from Santa Cruz (Santa Cruz, CA, USA). Microtubule-associated protein 1A/1B light chain 3B (LC-3) antibody (polyclonal, rabbit anti-human LC-3) was obtained from Abgent (San Diego, CA, USA). GAPDH expression level was used as an internal control for western blotting and the GAPDH antibody was purchased from Cell Signaling Technology Inc. The antibody against β-actin was purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA).

Cell viability analysis. Cell viability was analysed by the cell counting kit 8 (CCK-8) proliferation assay (Sigma-Aldrich, Inc., St. Louis, MO, USA) to determine the growth inhibition activity of flavopereirine on HCC cells. Briefly, 1×10^4 cells were seeded in 96-well plates, treated with flavopereirine (ChromaDex, Inc) at the indicated concentration, and incubated for 24 and 48 h at 37˚C in a humidified incubator. The absorbance values were read at 490 nm 2 h after the addition of CCK-8 reagent. The vehicle-treated cells were used as a control for normalisation. Three independent experiments were conducted and used to calculate the standard deviation (SD).

Cell-cycle analysis. To determine the cell-cycle profile after flavopereirine treatment, 1×10^5 cells were seeded in 6-well plates. Cells were starved, treated with flavopereirine for 24 and 48 h, then fixed with 100% methanol (Sigma-Aldrich, Inc.) for 24 h at 4˚C. The fixed cells were stained with propidium iodide (PI; 0.05 mg/ml, Sigma-Aldrich, Inc.) solution containing RNase (2 mg/ml) at 25˚C in the dark for 30 min. The DNA content was analysed by performing flow cytometry (FACSDiva; BD Biosciences) to determine the percentage of cells in each phase of the cell-cycle.
Detection of apoptosis by flow cytometric analysis. The percentage of apoptotic cells was determined after flavopereirine treatment. The cells were treated with the indicated concentration of flavopereirine for 24 and 48 h, then harvested and stained with Annexin-V and PI using an apoptosis detection kit (BioVision, Mountain View, CA, USA). Apoptotic cells were analysed by flow cytometry (FACSDiva; BD Biosciences).

Cell lysate preparation and western blotting. The cells were lysed and extracted with the M-PER mammalian protein extraction reagent (Thermo Scientific) containing 0.1% protease inhibitor cocktail (Cell Signaling Technology). Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA, USA) was used to determine the concentration of extracted protein. Forty μg of each sample was used and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), then electrotransferred onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). The membrane was blocked by incubation with 5% bovine serum albumin (Sigma-Aldrich) at 25˚C for 1 h, followed by probing with the primary specific antibody at 4˚C for 16 h. After incubation with a horseradish peroxidase-conjugated secondary antibody (Jackson

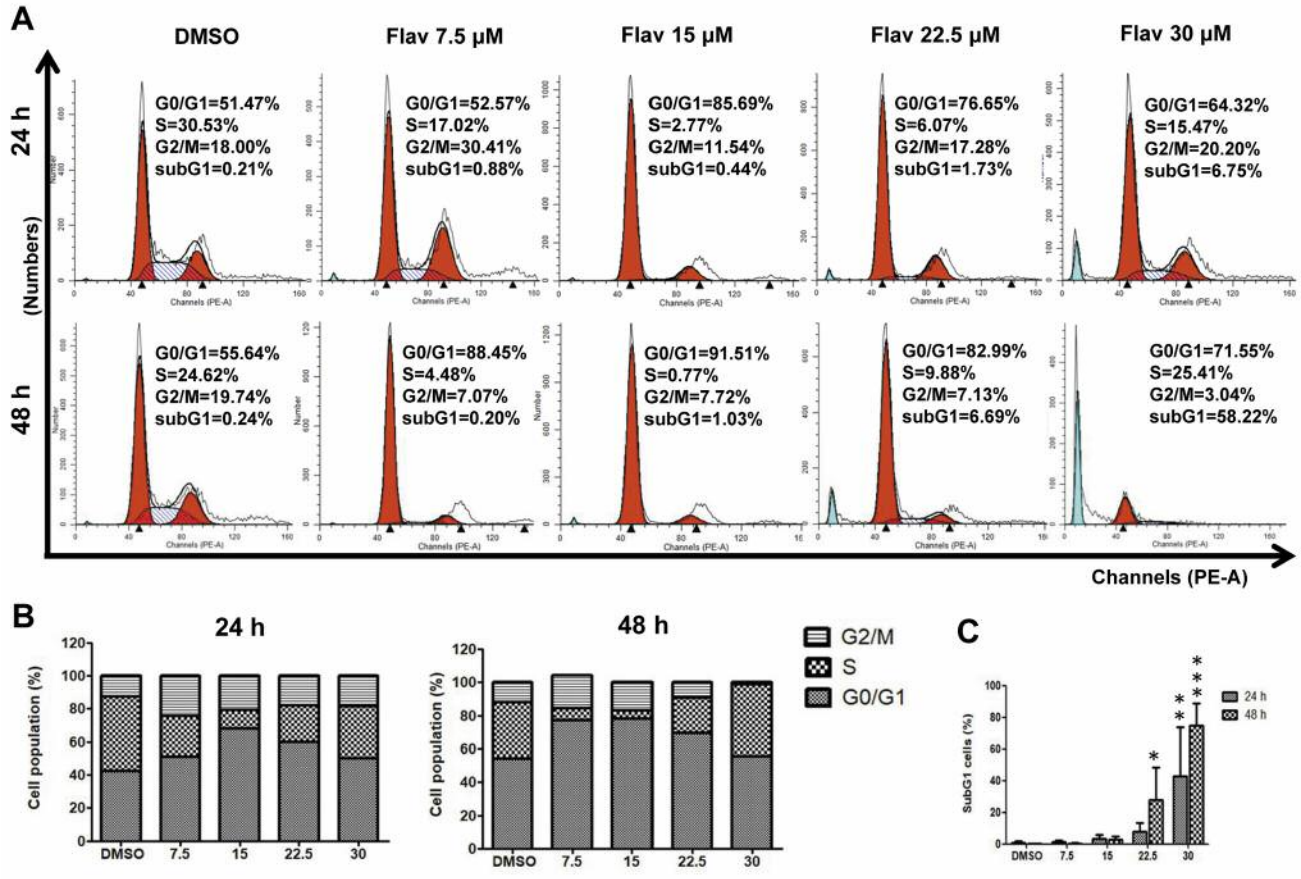

Figure 2. *Flavopereirine induces cell-cycle arrest in HepG2 cells. (A) HepG2 cells were treated with the indicated concentrations of flavopereirine for 24 or 48 h. Cell-cycle distribution was analysed by flow cytometry. Cell-cycle distribution and percentage of cell population are shown on the X-axis and Y-axis, respectively. (B) Cell-cycle distribution in (A) is represented as a histogram. (C) The sub-G_1 cell population was determined by flow cytometry. The X-axis represents different treatments of HepG2 cells and y-axis represents the percentage of sub-G_1 cells. Data are representative of at least three independent experiments and shown as mean±SD (*p<0.05; **p<0.01; ***p<0.001).*

ImmunoResearch Laboratories, West Grove, PA, USA) for 2 h at 25˚C, the protein expression was detected using an enhanced chemiluminescence horseradish peroxidase substrate detection kit (WBKLS0500, Millipore) and quantified using the UVP BioSpectrum 800 Imaging System (Cambridge, UK).

Statistical analysis. Statistical significance was determined using GraphPad Prism 7.0 software (GraphPad Software, San Diego, CA, USA). Data were analysed using one-way analysis of variance. All *in vitro* experiments were repeated three times and represented as mean±SD. A *p*-value less than or equal to 0.05 was considered statistically significant and indicated by *; **p-Value between 0.001 and 0.01; ***p-Value between 0.0001 and 0.001.

Results

Flavopereirine inhibits the growth of hepatocellular carcinoma (HCC) cells. To determine the cell survival ratio of HCC cells after flavopereirine treatment, HepG2 and Huh7 cells were treated with different concentrations of flavopereirine for 24 and 48 h. CCK-8 activity was then analysed to determine the growth inhibitory effects of flavopereirine on HepG2 and Huh7 cells. As shown in Figure 1, flavopereirine treatment inhibited the growth of HepG2 (Figure 1A) and Huh7 (Figure 1B) cells in a concentration and time-dependent manner. When compared with the control group treated with DMSO, flavopereirine had an IC_{50} of approximately 25 μM and 15 μM at 24 and 48 h, respectively.

Flavopereirine induces cell-cycle arrest in HCC cells. Interference of cell-cycle progression is one of the mechanisms for cell death caused by drug treatment. HepG2 cells showed G_0/G_1 cell-cycle arrest after flavopereirine treatment at a concentration of 7.5 and 15 μM for 24 and 48 h (Figure 2). At higher concentrations (22.5 and 30 μM), a decrease of G_0/G_1 distribution was found when compared

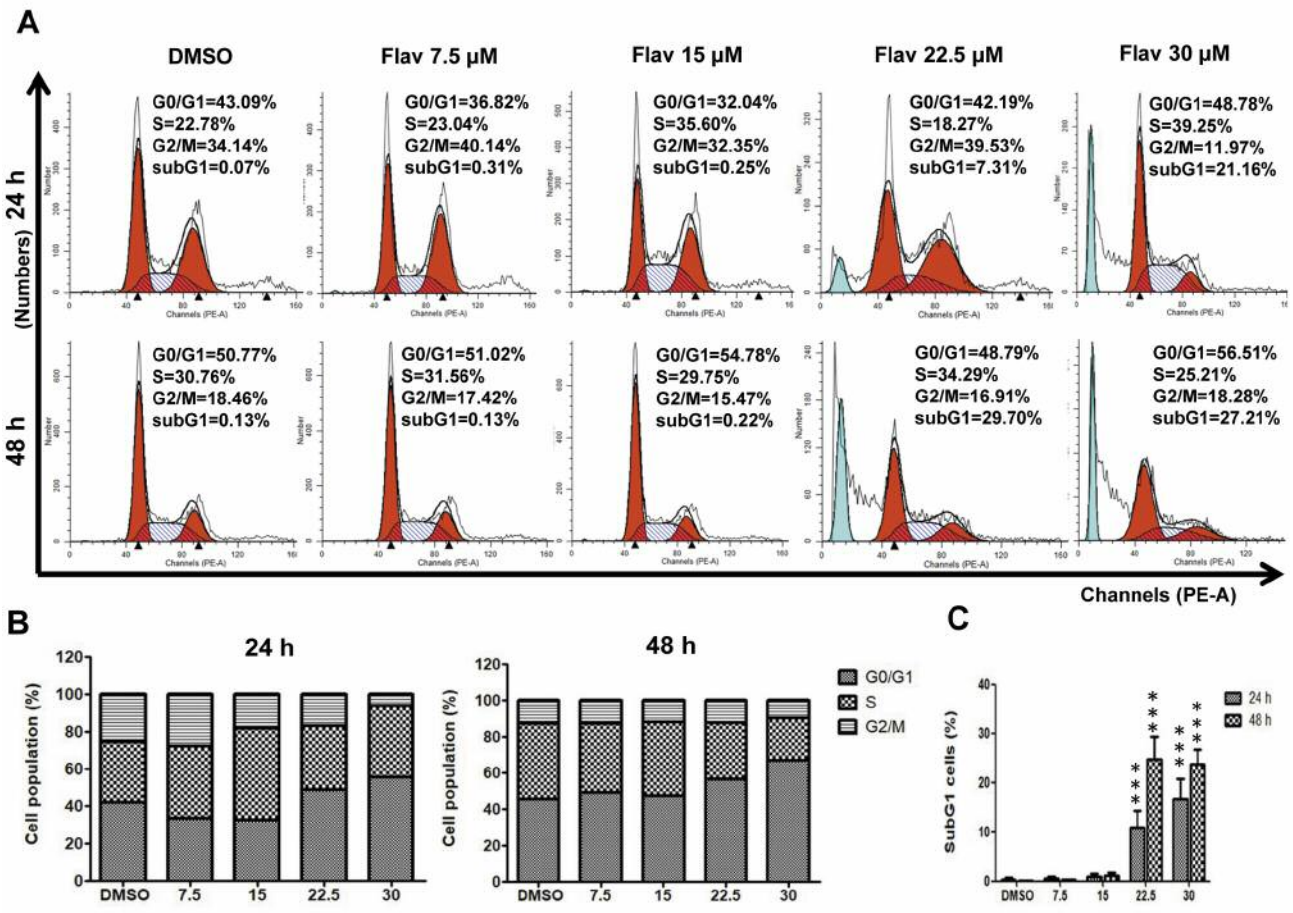

Figure 3. *Flavopereirine induces cell cycle arrest in Huh7 cells. (A) Huh7 cells were treated with the indicated concentrations of flavopereirine for 24 or 48 h. Cell cycle distribution was analysed by flow cytometry. (B) Cell cycle distribution in (A) is represented as a histogram. (C) The sub-G_1 cell population was determined after flow cytometry. The X-axis represents different treatments of Huh7 cells and y-axis represents the percentage of sub-G_1 cells. Data are representative of at least three independent experiments and shown as mean±SD (*p<0.05; **p<0.01; ***p<0.001).*

with the results of 15 μM, which may be due to cell death as an increase in sub-G_1 cell cells was observed in the flow cytometric analysis (Figure 2C). A similar effect was also found in Huh 7 cells. After application of flavopereirine, Huh7 cells showed a G_0/G_1 arrest in a concentration and time-dependent manner (Figure 3), with an increase of sub-G_1 cells at the higher concentration and longer exposure of flavopereirine. The flow cytometry analysis indicated that flavopereirine treatment induces G_0/G_1 arrest in HCC cells and causes cell death at higher concentrations.

Flavopereirine induced apoptosis and autophagy-related protein expression in HepG2 and Huh7 cells. Flow cytometric analysis was performed to evaluate apoptosis after flavopereirine treatment using Annexin-V/PI double staining. As shown in Figure 4 and 5, flavopereirine treatment caused an increase in Annexin-V staining in a concentration-dependent and time-dependent manner. The apoptotic ratio in HepG2 cells increased from 14.1% to 41.5% at flavopereirine concentration from 7.5 to 30 μM at 24 h, and 11.5% to 79.3% at 48 h (Figure 4). Similarly, the apoptotic ratio in Huh7 cells increased from 4.3% to 30.9% at flavopereirine concentration from 7.5 to 30 μM at 24 h, and 15.9% to 91.7% at 48 h (Figure 5).

Apoptosis is characterised by a cascade of caspase cleavage and activation (17). We then determined which caspases were involved in apoptotic cell death after flavopereirine treatment by western blotting. The cleaved form of caspase 3 and poly(ADP)-ribose polymerase (PARP) or decreased preform-PARP were detected after flavopereirine treatment in HepG2 and Huh7 cells. We also found that the cleaved form of caspase 8 and caspase 9 increased after flavopereirine treatment in HepG2 cells but not so clear in Huh7 cells (Figure 6), indicating that

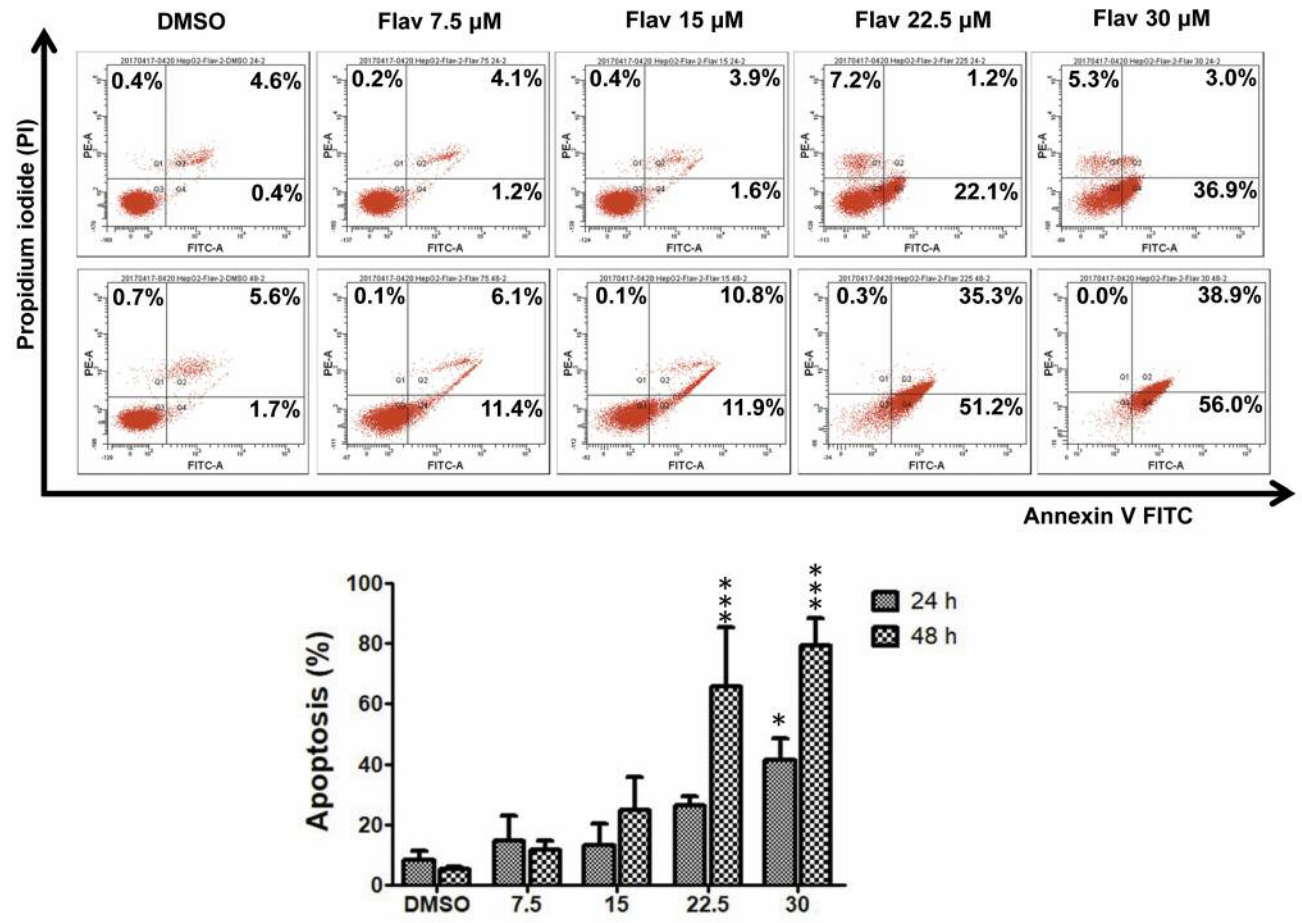

Figure 4. *Flavopereirine induces apoptosis in HepG2 cells. (A) HepG2 cells were treated with the indicated concentrations of flavopereirine for 24 or 48 h. Apoptotic cells were determined by PI and Annexin-V staining and analysed by flow cytometry. (B) The X-axis represents different treatments of HepG2 cells and y-axis represents the percentage of apoptotic cells. Data are representative of at least three independent experiments and are shown as means±SD (*p<0.05; **p<0.01; ***p<0.001).*

flavopereirine treatment resulted in apoptotic cell death involving both intrinsic and extrinsic pathways in HCC cells.

A cytoplasmic vacuole was present in flavopereirine-treated cells, indicating that autophagy may be induced after flavopereirine treatment. During autophagy, cytoplasmic LC3-I is conjugated to phosphatidylethanolamine to form LC3-II. A cargo adaptor protein, p62 protein, interacts with the target protein and p62 for degradation in autophagy (18, 19). Therefore, a change in LC-3 II and p62 protein expression is an indicator of the initiation of autophagy. We then determined the LC-3 II expression level in HepG2 and Huh7 cells by western blotting after flavopereirine treatment. As shown in Figure 6, LC-3 II expression increased after flavopereirine treatment in HepG2 and Huh7 cells, indicating that flavopereirine affected autophagy in HCC cells. Taken together, flavopereirine treatment induced apoptosis and affected autophagy in treated HCC cells.

Discussion

HCC is the most common primary malignancy of the liver. It is imperative to develop new drugs for HCC treatment. In the current study, we evaluated the effects of flavopereirine treatment on HCC cells, showing that flavopereirine exhibits cytotoxicity in HepG2 and Huh7 cells. Flavopereirine induces G_0/G_1 cell-cycle arrest and apoptosis in HCC cells involving both intrinsic and extrinsic pathways. An alteration of autophagy-related protein expression was also found after flavopereirine treatment. Taken together, the current findings indicate that flavopereirine is a potential drug for HCC treatment.

It has been demonstrated that the natural compounds β-carboline alkaloids have anticancer activity by targeting cell division in cancer cells (20). Flavopereirine, a β-carboline alkaloid, has also been reported to exhibit anti-growth activity

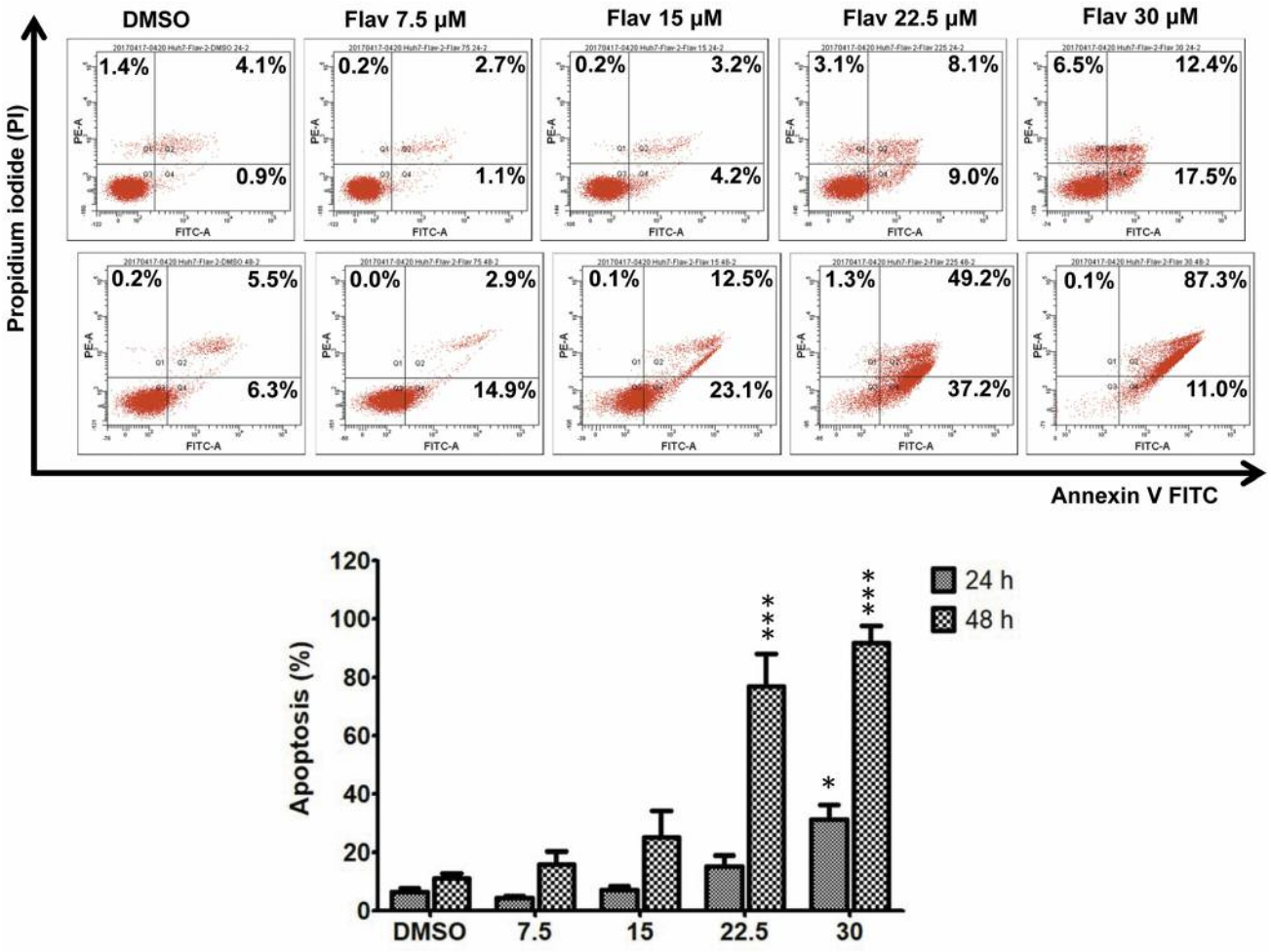

Figure 5. *Flavopereirine induces apoptosis in Huh7 cells. (A) Huh7 cells were treated with the indicated concentrations of flavopereirine for 24 or 48 h. Apoptotic cells were determined by PI and Annexin-V staining and analysed by flow cytometry. (B) The X-axis represents different treatments of Huh7 cells and y-axis represents the percentage of apoptotic cells. Data are representative of at least three independent experiments and are shown as means±SD (*p<0.05; **p<0.01; ***p<0.001).*

by interfering with cell-cycle progression in colon cancer and breast cancer cells. Flavopereirine induces G_2/M cell-cycle arrest in colon cancer cells (14), increasing G_0/G_1 cell-cycle phase in breast cancer cells (15). P53 protein expression plays a key role in flavopereirine-mediated cell death in colon cancer cells, as a loss of P53 protein resulted in more resistance to flavopereirine treatment (14). HepG2 expresses wild-type and functional P53 protein, whereas Huh7 carries a P53 protein mutation at codon 220 which is aberrantly stable but transcriptionally inactive (21). In our study, we found that HepG2 and Huh7 cells showed a similar response at IC_{50} and cell-cycle distribution, and caspase activation after flavopereirine treatment. The role of P53 protein in flavopereirine mediated cytotoxicity in HCC cells needs to be further determined.

Apoptosis can be activated by intrinsic or extrinsic caspases pathways (22), with both pathways being involved in flavopereirine mediated apoptosis in HepG2 and Huh7 cells, as both cleavage of caspase 8 and caspase 9, which represent extrinsic and intrinsic pathway activation, were detected in western blotting analysis. The activation of both caspases pathways was also shown in flavopereirine treated CRC and breast cancer cell lines (14, 15), suggesting that flavopereirine triggers cell apoptosis by activating both caspase pathways in cancer cells.

Flavopereirine has been reported to regulate autophagy by blocking LC3-II accumulation in autophagosomes in breast cancer cells (16). Autophagy is a catabolic process and contributes to liver homeostasis under normal and stressed conditions (23, 24). The high expression of autophagy-related

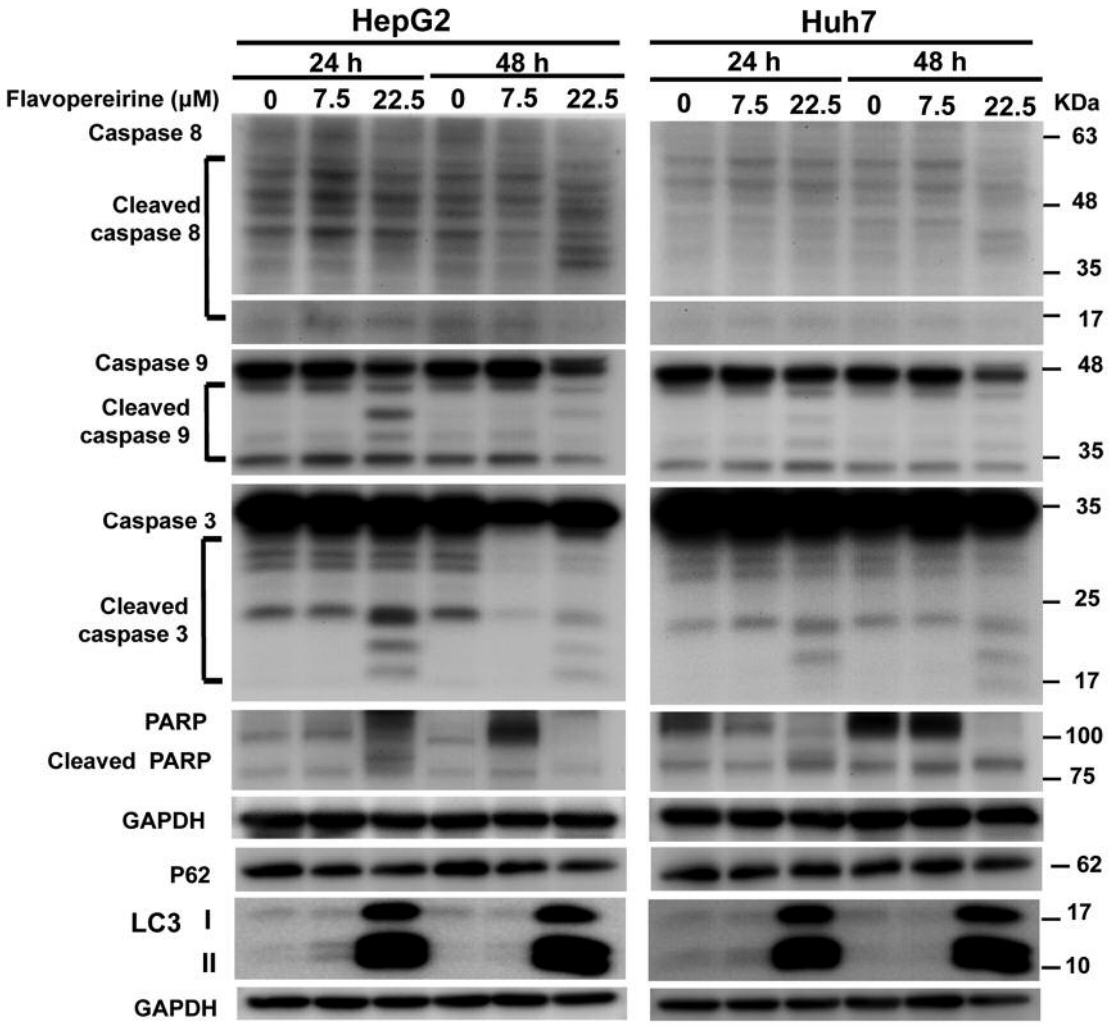

Figure 6. *Flavopereirine induces apoptosis and autophagy-related protein expression in HCC cells. HepG2 and Huh7 cells were treated with the indicated concentrations of flavopereirine for 24 h and 48 h. Expression levels of caspase 8, caspase 9, caspase 3, PARP, p62 protein and LC3 proteins were analysed by western blotting.*

LC3 predicts recurrence and poor prognosis in HCC patients (25, 26). Therefore, autophagy may play a crucial role in HCC. We also detected LC3-II accumulation by western blotting after flavopereirine treatment in HCC cells, suggesting that flavopereirine may interfere autophagy pathways in HCC cells.

Taken together, we showed that flavopereirine exhibits cytotoxicity in both HepG2 and Huh7 cells by triggering apoptosis and regulating autophagy, hence, may constitute a potential drug for the treatment of HCC. Further studies are needed to investigate the application of flavopereirine to treat HCC in the future.

Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

SYC designed the research and collected data, CNC prepared materials and helped with the literature search, HYH performed the experiments. CYF analyzed and interpreted the data. All authors read and approved the final manuscript.

Acknowledgements

The present study was supported by the Ditmanson Medical Foundation Chiayi Christian Hospital [grant number R107-008].

References

1 Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A and Roberts LR: A global view of hepatocellular carcinoma: Trends, risk,

prevention and management. Nat Rev Gastroenterol Hepatol *16(10)*: 589-604, 2019. PMID: 30231359. DOI: 10.1200/EDBK_200939

2 Forner A, Reig M and Bruix J: Hepatocellular carcinoma. Lancet *391(10127)*: 1301-1314, 2018. DOI: 10.1016/S0140-6736(18)30010-2

3 Mak LY, Cruz-Ramón V, Chinchilla-López P, Torres HA, LoConte NK, Rice JP, Foxhall LE, Sturgis EM, Merrill JK, Bailey HH, Méndez-Sánchez N, Yuen MF and Hwang JP: Global epidemiology, prevention, and management of hepatocellular carcinoma. Am Soc Clin Oncol Educ Book *38:*262-279, 2018. PMID: 30231359. DOI: 10.1200/EDBK_200939

4 Villanueva A: Hepatocellular carcinoma. N Engl J Med *380(15)*: 1450-1462, 2019. DOI: 10.1056/NEJMra1713263

5 Tabrizian P, Jibara G, Shrager B, Schwartz M and Roayaie S: Recurrence of hepatocellular cancer after resection: Patterns, treatments, and prognosis. Ann Surg *261(5)*: 947-955, 2015. PMID: 25010665. DOI: 10.1097/SLA.0000000000000710

6 Zhang T, Zhang L, Xu Y, Lu X, Zhao H, Yang H and Sang X: Neoadjuvant therapy and immunotherapy strategies for hepatocellular carcinoma. Am J Cancer Res *10(6)*: 1658-1667, 2020. PMID: 32642282.

7 Lohitesh K, Chowdhury R and Mukherjee S: Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: An insight. Cancer Cell Int *18*: 44, 2018. PMID: 29568237. DOI: 10.1186/s12935-018-0538-7

8 Marin JJG, Macias RIR, Monte MJ, Romero MR, Asensio M, Sanchez-Martin A, Cives-Losada C, Temprano AG, Espinosa-Escudero R, Reviejo M, Bohorquez LH and Briz O: Molecular bases of drug resistance in hepatocellular carcinoma. Cancers (Basel) *12(6)*: 1663, 2020. PMID: 32585893. DOI: 10.3390/cancers12061663

9 Reina M, Ruiz-Mesia W, López-Rodríguez M, Ruiz-Mesia L, González-Coloma A and Martínez-Díaz R: Indole alkaloids from geissospermum reticulatum. J Nat Prod *75(5)*: 928-934, 2012. PMID: 22551062. DOI: 10.1021/np300067m

10 Steele JC, Veitch NC, Kite GC, Simmonds MS and Warhurst DC: Indole and beta-carboline alkaloids from geissospermum sericeum. J Nat Prod *65(1)*: 85-88, 2002. PMID: 11809075. DOI: 10.1021/np0101705

11 da Silva ESJV, Cordovil Brigido HP, Oliveira de Albuquerque KC, Miranda Carvalho J, Ferreira Reis J, Vinhal Faria L, Coelho-Ferreira M, Silveira FT, da Silva Carneiro A, Percário S, do Rosário Marinho AM and Dolabela MF: Flavopereirine-an alkaloid derived from geissospermum vellosii-presents leishmanicidal activity *in vitro*. Molecules *24(4)*:785 , 2019. PMID: 30795632. DOI: 10.3390/molecules24040785

12 Beljanski M and Beljanski MS: Selective inhibition of *in vitro* synthesis of cancer DNA by alkaloids of beta-carboline class. Exp Cell Biol *50(2)*: 79-87, 1982. PMID: 7075859. DOI: 10.1159/000163131

13 Beljanski M, Crochet S and Beljanski MS: Pb-100: A potent and selective inhibitor of human bcnu resistant glioblastoma cell multiplication. Anticancer Res *13(6a)*: 2301-2308, 1993. PMID: 8297150.

14 Li JM, Huang YC, Kuo YH, Cheng CC, Kuan FC, Chang SF, Lee YR, Chin CC and Shi CS: Flavopereirine suppresses the growth of colorectal cancer cells through p53 signaling dependence. Cancers (Basel) *11(7)*: 1034, 2019. PMID: 31336690. DOI: 10.3390/cancers11071034

15 Yeh HT, Tsai YS, Chen MS, Li YZ, Lin WC, Lee YR, Tseng YS and Sheu SM: Flavopereirine induces cell cycle arrest and apoptosis *via* the akt/p38 mapk/erk1/2 signaling pathway in human breast cancer cells. Eur J Pharmacol *863:* 172658, 2019. PMID: 31518562. DOI: 10.1016/j.ejphar.2019.172658

16 Chen MS, Yeh HT, Li YZ, Lin WC, Lee YR, Tseng YS and Sheu SM: Flavopereirine inhibits autophagy *via* the akt/p38 mapk signaling pathway in mda-mb-231 cells. Int J Mol Sci *21(15)*: 5362, 2020. PMID: 32731544. DOI: 10.3390/ijms21155362

17 Shi Y: Mechanisms of caspase activation and inhibition during apoptosis. Mol Cell *9(3)*: 459-470, 2002. PMID: 11931755. DOI: 10.1016/s1097-2765(02)00482-3

18 Lee YK and Lee JA: Role of the mammalian atg8/lc3 family in autophagy: Differential and compensatory roles in the spatiotemporal regulation of autophagy. BMB Rep *49(8)*: 424-430, 2016. PMID: 27418283. DOI: 10.5483/bmbrep.2016.49.8.081

19 Liu WJ, Ye L, Huang WF, Guo LJ, Xu ZG, Wu HL, Yang C and Liu HF: P62 links the autophagy pathway and the ubiqutin-proteasome system upon ubiquitinated protein degradation. Cell Mol Biol Lett *21*: 29, 2016. PMID: 28536631. DOI: 10.1186/s11658-016-0031-z

20 Ahmad I, Fakhri S, Khan H, Jeandet P, Aschner M and Yu ZL: Targeting cell cycle by β-carboline alkaloids *in vitro*: Novel therapeutic prospects for the treatment of cancer. Chem Biol Interact *330*: 109229, 2020. PMID: 32835667. DOI: 10.1016/j.cbi.2020.109229

21 Bressac B, Galvin KM, Liang TJ, Isselbacher KJ, Wands JR and Ozturk M: Abnormal structure and expression of p53 gene in human hepatocellular carcinoma. Proc Natl Acad Sci U S A *87(5)*: 1973-1977, 1990. PMID: 2155427. DOI: 10.1073/pnas.87.5.1973

22 McIlwain DR, Berger T and Mak TW: Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol *7(4)*: a026716, 2015. PMID: 25833847. DOI: 10.1101/cshperspect.a026716

23 Cui J, Gong Z and Shen HM: The role of autophagy in liver cancer: Molecular mechanisms and potential therapeutic targets. Biochim Biophys Acta *1836(1)*: 15-26, 2013. PMID: 23428608. DOI: 10.1016/j.bbcan.2013.02.003

24 Allaire M, Rautou PE, Codogno P and Lotersztajn S: Autophagy in liver diseases: Time for translation? J Hepatol *70(5)*: 985-998, 2019. PMID: 30711404. DOI: 10.1016/j.jhep.2019.01.026

25 Lin CW, Chen YS, Lin CC, Lee PH, Lo GH, Hsu CC, Hsieh PM, Koh KW, Chou TC, Dai CY, Huang JF, Chuang WL, Chen YL and Yu ML: Autophagy-related gene lc3 expression in tumor and liver microenvironments significantly predicts recurrence of hepatocellular carcinoma after surgical resection. Clin Transl Gastroenterol *9(6)*: 166, 2018. PMID: 29961754. DOI: 10.1038/s41424-018-0033-4

26 Meng YC, Lou XL, Yang LY, Li D and Hou YQ: Role of the autophagy-related marker lc3 expression in hepatocellular carcinoma: A meta-analysis. J Cancer Res Clin Oncol *146(5)*: 1103-1113, 2020. PMID: 32157437. DOI: 10.1007/s00432-020-03174-1

Received October 20, 2020
Revised November 3, 2020
Accepted November 13, 2020