Research article
Eurasian J Bio Chem Sci, 2(Suppl 1): 115-119, 2019

EJBCS

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.org.tr/ejbcs

The effect of neopterin alone or in combination with doxorubicin, cisplatin and vitamin C on the viability of different hepatocellular carcinoma cell lines

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Received : 06/08/2019
Accepted : 21/12/2019

Abstract: Hepatocellular carcinoma (HCC) is inflammation-related cancer, and the 4th leading cause of death globally. Neopterin is an instant biomarker of the cellular immune response and belongs to the pteridine class. The levels of neopterin are believed to be very closely linked with the tumor size in patients with HCC. Various studies have proven that neopterin can modulate the cellular oxidant-antioxidant balance, thus causing cell toxicity. In this study, we investigated the cytotoxic effects of neopterin alone and in combination with two of the known cytotoxic agents of doxorubicin and cisplatin together with vitamin C, a well-known antioxidant agent. SNU-449, Hep3B, Mahlavu, and PLC/PRF/5 HCC cell lines were used in this study. Our results showed that increasing concentrations of neopterin does not have any significant effects on the cytotoxicity while as expected the three other agents decrease the viability of all subjected cell lines. SNU-449 is the most resistant HCC cell line among others. Considering the effectivity of combinational therapy in cancer patients, the effect of various combinations of neopterin with doxorubicin, cisplatin and vitamin C on the viability of the most resistant cell line of SNU-449 has been investigated. We found that the addition of cisplatin to the combination of neopterin and vitamin C, causes a greater decrease in cell viability of SNU-449 cells compared to the dual therapy with neopterin and vitamin C, while the addition of doxorubicin to the same dual therapy, leads to a decrease in the effectivity of it.

Keywords: HCC, neopterin, doxorubicin, cisplatin, vitamin C, combinational therapy

1. Introduction

Hepatocellular carcinoma (HCC) is inflammation-related liver cancer, with a high recurrence rate and poor long-term survival. HCC is considered as the 4th most common cancer, and the 2nd leading cause of death amongst cancer cases, globally. (Mazzanti et al.,2016) Conventional cytotoxic chemotherapies such as the use of sorafenib, cisplatin, and doxorubicin have not provided clinical benefits or prolonged survival for patients with advanced HCC. Beside its complications; surgery, including transplantation and resection, remains the most effective treatment for HCC. (Daher et al., 2018) In a recent study, it has been suggested that vitamin C preferentially kills HCC cancer stem cells. (Lv et al., 2018) Current advances have shown that combinational therapy may be more effective than using just a single chemotherapeutic approach in cancer management. But even these combination therapies are effective only on a small number of tumors, and the responses are not promising. (Jindal et al., 2019) Therefore, scientific researches are now focusing on discovering novel chemotherapeutics with molecular targets for the management of HCC.

It is very well known that inflammation plays an important role in pathogenesis and progression of HCC by mediating cellular survival and metastasis. Studies have revealed that the inflammation status and relevant molecules are becoming popular targets of drug discovery. (Capece et al., 2013) (Lewis and Merched, 2014) Recently, attention has been drawn to the role of neopterin in the already established relation between inflammation and HCC. It is believed that, the levels of neopterin are very closely linked with the tumor size in these patients. (Antoniello et al., 1992) (Godai et al., 1994)

Neopterin and its derivatives are metabolites of guanosine triphosphate and are produced by the human monocyte-derived macrophages upon stimulation with γ-interferon. Various studies have suggested that neopterin derivatives belonging to pteridine family can modulate the redox balance of the biological systems. (Huber et al., 1984) Generally, the reduced pteridine species are scavengers of

free radicals while the oxidized forms act as weak to strong enhancers of oxidative stress. For instance, neopterin derivatives can play a substituent role as an immune modulator in nitric oxide generation and other oxygen radical-mediated processes. 7,8-Dihydronepterin has shown to be a potential free-radical scavenger both in-vivo and in-vitro studies. (Hoffmann et al., 2003) However, besides the redox state of neopterin derivatives other factors such as the composition of the culturing medium, can change the type of biochemical role played by neopterin derivatives in biological systems. Some studies showed that neopterin activates tumor necrosis factor-alpha-induced apoptosis through oxygen radical-mediated processes. (Hoffmann et al., 1998) However, the effects of neopterin on the HCC cells are not yet understood. Given that combinational therapies may have higher effectivity in cancer management, in this study; four different HCC cell lines of SNU-449, Hep3B, Mahlavau and PLC/PRF/5 have been subjected to cellular cytotoxicity test (MTT analysis) after being treated with neopterin and its various combination with chemotherapeutics such as doxorubicin, cisplatin and a well known antioxidant, vitamin C.

2. Materials and Method

2.1. Cell Culture and chemicals

HCC cell lines (SNU-449, Hep-3B, Mahlavau and PLC/PRF/5) were kindly provided by Prof. Dr. Mehmet Öztürk (Izmir Biomedicine and Genome Centre). All cell lines were maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 2 mM L-glutamine, 100 mg/mL streptomycin and 1X NEAA, at 5% CO at 37 °C. Neopterin (25 µM-500 µM), doxorubicin (2 µM and 60 µM), cisplatin (10 µM and 50 µM) and vitamin C (1mM) treatments were carried out along 48-h for all the viability experiments.

2.2. MTT assay

Cells were seeded in a 96 well plate. After overnight incubation, they were treated with different concentrations of neopterin, doxorubicin, cisplatin and vitamin C containing medium. For MTT, following 48-h treatment, 0.5 mg/mL MTT (Thiazolyl Blue Tetrazolium Bromide) was added to the medium. After the formation of formazan crystals, medium was removed, and formazan crystals were dissolved with 100 µL DMSO. Absorption of solution was measured at 570 nm. The cell viability was calculated as viable cell percentage.

2.3. Statistical analysis

Experiments have been carried out three times, independently. The results were reported as mean ± SE for three repetitive analysis. One-way ANOVA test was used for data analysis. $p < 0.05$ was considered as statistically significant. All the analyses were performed using GraphPad Prism 5 software.

3. Results

3.1. The effect of different concentration of neopterin on the viability of different HCC cell lines

All the HCC cell lines, SNU-449, Hep3B, Mahlavu and PLC/PRF/5, have all been mixed with neopterin in a concentration range of 25 µM-500 µM. As shown in Figure 1, within the chosen range, none of the neopterin concentrations had any statistically significant effects on the proliferation and viability of any of the subjected cell lines.

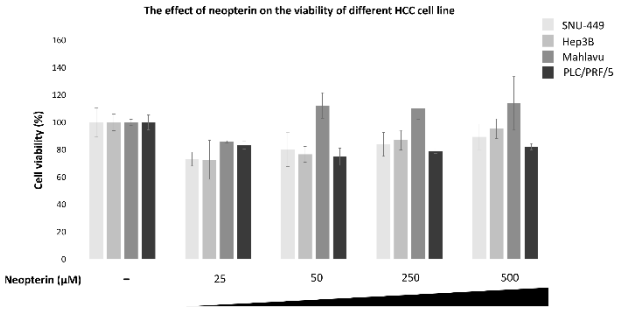
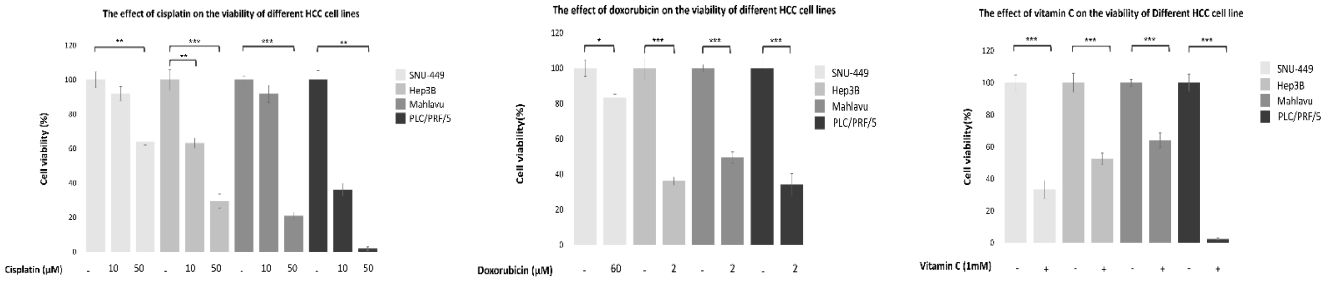


Figure 1. The effect of increasing neopterin concentrations (25µM-500 µM) on the viability of different HCC cell lines.

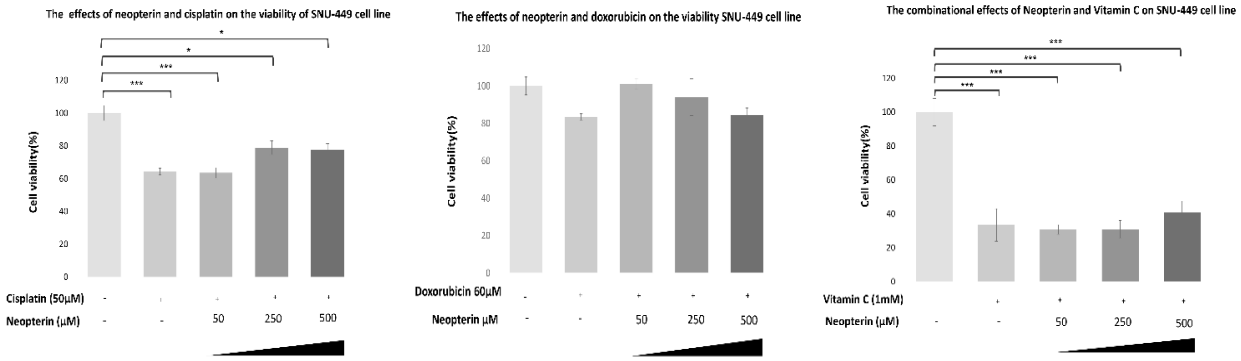
3.2. The effect of different concentrations of cisplatin, doxorubicin and vitamin C on the viability of HCC cell lines

Based on the American Cancer Society, cisplatin and doxorubicin are among the most commonly used chemotherapeutics in the treatment of liver cancer. Scientists have recently reported the possible cytotoxic effect of vitamin C on HCC cancer stem cells (Lv et al. 2018). In this study the effect of different concentrations of cisplatin (10µM and 50µM), doxorubicin (2µM and 60µM), and vitamin C (1mM) treatment on the viability of SNU-449, Hep-3B, Mahlavau and PLC/PRF/5, has been investigated. A drastic decrease in the viability of all the cell lines has been noticed with 50 µM cisplatin (***$p<0.0001$, **$p<0.001$). On a sensitivity scale, SNU-449 was the least and PLC/PRF/5 was the most sensitive cell line to doxorubicin treatment (***$p<0.0001$, *$p<0.01$, respectively). As expected, vitamin C caused a decrease in cell viability of SNU-449, Mahlavau and PLC/PRF/5 (***$p<0.0001$) (Fig. 2a, 2b, 2c).



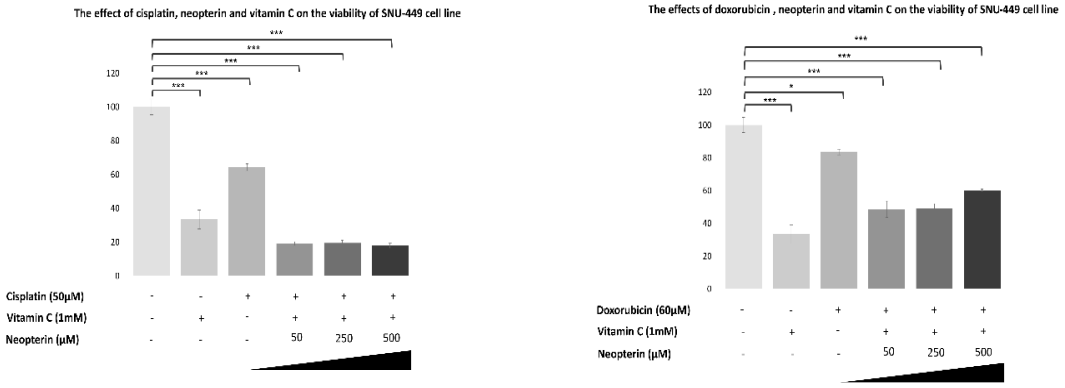
(a) (b) (c)

Figure 2. The effect of (a) cisplatin (b) doxorubicin* (c) vitamin C on the viability of differrent HCC cell lines. *(because of its resistance only SNU-449 was treated with 60 μM doxorubicin, while other cell lines were treated with 2 μM doxorubicin).



(a) (b) (c)

Figure 3. The combinational effect of neopterin with (a) cisplatin, (b) doxorubicin, (c) vitamin C. on the viability of SNU-449 cell lines



(a) (b)

Figure 4. The combination of vitamin C and neopterin with (a) cisplatin and (b) doxorubicin. on the viability of SNU-449 cell line

3.3. The effect of combinational exposure of neopterin with doxorubicin, cisplatin and vitamin C on SNU-449 cell line.

Even though the SNU-449 cells have sensitivity to vitamin C, it is still resistant to doxorubicin and cisplatin. In search of new therapeutic approaches, the effect of neopterin on the viability of SNU-449 as in combination with doxorubicin, cisplatin and vitamin C has been investigated. Our results revealed that neopterin significantly protects the cells from the cytotoxicity of cisplatin treatment especially at higher concentration (***$p<0.0002$, *$p<0.01$) (Fig. 3a). However, neopterin did not potentiate the cytotoxic effects of neither vitamin C nor doxorubicin (Fig. 3b, 3c)

3.4. The effect of cisplatin and doxorubicin in combination with vitamin C and neopterin on SNU-449 cell line.

Eventually, the effect of cisplatin in combination with vitamin C and neopterin on the viability of SNU-449 was analyzed. The results revealed that the addition of cisplatin enhances the cytotoxic potency compared to double combinational therapy with vitamin C and neopterin (***$p<0.001$) (Fig. 3c and Fig. 4a). With the same logic the effect of doxorubicin in combination with vitamin C and neopterin on SNU-449 has been investigated. When doxorubicin was added to the combination of vitamin C and neopterin, a decrease in cytotoxic potency compared to double combinational therapy has been found (***$p<0.001$,*$p<0.01$) (Fig. 4b and 3b).

4. Discussion

Although previous studies have reported neopterin to be an inducer of programmed cell death via the mediation of oxidative stress, to the best of our knowledge this is the only study analyzing the effect of neopterin and its combinations on the viability of different HCC cell lines. (Hoffmann et al., 2003) (Baier-bitterlich et al., 1995) Based on the American cancer society, cisplatin and doxorubicin are two of the main chemotherapeutics used in the management of liver cancer. Moreover, some studies have also shown that vitamin C has the ability to induce anticancer activity in various cell lines. (Alexander et al., 2013)(Yiang et al., 2014) Our results showed that neopterin alone has no significant effect on the viability but vitamin C significantly decreases the viability of all the analyzed HCC cell lines. Besidesits sensitivity to vitamin C treatment, SNU-449 still remained the most resistant analyzed cell line of this study. Due to the growing focus on thecombinational therapy of cancer the effect of neopterin in various combination with cisplatin, doxorubicin and vitamin C on the viability of the resistant SNU-449cell lines have been then investigated. In double combinational therapy with neopterin and cisplatin, it has been noticed that at high concentrations neopterin protects the SNU-449 cells against the cytotoxic effects of cisplatin. Interestingly, the double combinational therapy of neopterin and vitamin C still, lead to the maximum amount of decrease in cell viability of SNU-449. However, this decreased in cell viability did not have any significant difference compared to the single therapy with vitamin C. For this reason, the triple combinational therapy of doxorubicin and cisplatin in combination with neopterin and vitamin C has been analyzed, independently. The addition of cisplatin lead to an increased cytotoxic potency, while the addition of doxorubicin caused a decrease in the cytotoxic potency of vitamin C and neopterin combinational therapy. Based on our results, neopterin can show a dual activity both as inducer and inhibitor of apoptosis depending on the cell line and the cytotoxic agents that it is used in combination with. In further studies, we are planning to analyze the underlying molecular mechanism of the effects of neopterin with a focus on the signaling pathways.

5. Conclusions

The results of this study, highlights the dual activity of neopterin both as hepatoprotective and hepatotoxic agent dependent on the type of cell line and the combinational therapy. However, further in detailed studies are required to fully clarify this dual biological activity of neopterin, and its potentail future use in HCC management.

Acknowledgments

This research received no external funding.

Authors' contributions:

I.K and G.S conceived and designed the experiment; I.K, N.Z, M.N,Y.S and G.S performed the experiment, I.K and N.Z analyzed the data, I.K and N.Z wrote the manuscript.

Conflict of interest disclosure:

There was no conflict of interest.

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