The Effect of Curcumin in Combination Chemotherapy with 5-FU on non-Malignant Fibroblast Cells

Hassan Sarkhosh¹, Reza Mahmoudi², Mohammadreza Malekpour³, Zohreh Ahmadi⁴, Azim Akbarzadeh Khiyavi⁵

¹Department of Medicine, Qom Islamic Azad University, Qom, Iran. ²Department of Toxicology, Faculty of Pharmacy, Islamic Azad University, Shahreza, Iran. ³Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran. ⁴MSc Student of Health Education and Health Promotion, Faculty of Health, Qom University of Medical Sciences, Qom, Iran. ⁵Department of Pilot Nanobiotechnology, Pasteur Institute of Iran, Tehran, Iran.

Abstract

Background: Curcumin has long been known to possess therapeutic properties and recent research have shown that it can inhibit malignant cell proliferation in vivo and in vitro. However, studies on combination therapy with curcumin and chemotherapeutic drugs are so limited. Methods: With this regard, we studied the effect of curcumin on the toxicity of 5-Flouracil (5-FU) in the treatment of normal fibroblasts in vitro using L929 (nonmalignant fibroblast cell) cell line. Curcumin in the doses of 5 and 15 µM was used. First control groups treated to curcumin, alone while second control groups received the chemotherapeutic drug, separately. Experimental groups received curcumin in combination with 5-Flouracil. Cell viability was measured after 24, 48, and 72h through MTT method. Statistical differences were analyzed by ANOVA test. Results: At all studied times in combination cases (curcumin+5-FU) with increasing concentration of curcumin, toxicity was decreased. Indeed, curcumin in combination with 5-Fu in low concentration inhibited the effect of 5-FU (p<0.05). Conclusion: It can be concluded that curcumin in combination therapy with 5-FU may induce lower toxicity in normal cells and reduce possible side effects.

Keywords: Curcumin- 5-Flouracil- Fibroblast cells

Introduction

Cancer considered as the major health problem in modern medicine which is related to the high rate of mortality after cardiovascular diseases in enormous countries [1]. Today, whether in industry [2, 3-4] or medicine [1] applications, the use of nanotechnology has become an important tool to improve the efficiency and the health states. Indeed, there are numerous surveys which investigate the role of the tumor microenvironment in cancer development [5-8]. Fibroblasts in the tumor stroma are known with their crucial function in carcinogenesis especially in the initiation of epithelial tumor progression. Indeed these cells surrounding the cancerous tissue form a separate microenvironment [2]. Based on the previous report investigate the possible response of patient toward chemotherapeutics is an important issue in cancer-related treatments [9]. Indeed, The major element indefinite drug delivery strategies are precise drug delivery to the targeted organ with optimal concentration with minimal toxicity and maximum efficiency of the drug [10]. In this regards, Chemotherapy is a major treatment option in various cancers [11]. Though the most considerable effects of chemotherapy for cancer therapy, the toxicity impacts of chemotherapy exerted organ damages include liver and kidney injury, immunosuppressant, and etc [13]. Recently, investigated the novel, less toxic and, effective therapeutic strategies has been the main issues in advanced cancer treatments [13]. Certainly, Chemotherapy non-toxic agents could be an interesting attitude for decreased the cancer-related stigma [14]. Besides, new cytotoxic agents which definitely destroy the cancer cells and
Materials and Methods

Materials

Mouse fibroblast cell line (L929) was obtained from Pasteur Institute-Iran (national cell bank of Pasteur Institute) and curcumin form MERK Company. MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], DMSO, RPMI 1640 (Roswell Park Memorial Institute), Fetal Bovine serum (FBS), phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO). Ferric reducing/antioxidant power (FRAP) photometric in vitro assay kit obtained from East Sage Holding Research Company.

Cell culture and treatment

L929 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin in a humidified 95% air/5% CO\textsubscript{2} incubator at 37°C. Cells were allowed attaching for 12 h before treatment. Curcumin (98% purity) was dissolved in DMSO (Dimethyl Sulfoxide), as a 10 mg/ml stock solution, and stored at -20°C in a light protected cover. Curcumin was stored at -20°C in a light protected cover. Curcumin was stored at -20°C in a light protected cover. Curcumin was used in combination with 5-fluorouracil

Results

MTT analysis shows that curcumin exerted lower cytotoxic effects in compared to 5-FU in all times and concentrations in compared to 5-FU (P<0.05). At all times in combination cases (curcumin+5-FU) with increasing concentrations of curcumin, toxicity was decreased. 5-FU toxicity at all times was significant. Indeed, curcumin in combination with 5-Fu in low concentration inhibited the effect of 5-FU (p<0.05). The cellular viability in different

Table 1. The Results of Cellular Viability Assay which Presented as Mean ± Standard Deviation by MTT Method in L-929 Cell Lines

<table>
<thead>
<tr>
<th>Groups</th>
<th>L-929 relative cell viability, 24 h</th>
<th>L-929 relative cell viability, 48 h</th>
<th>L-929 relative cell viability, 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin (5 μM)</td>
<td>3.013±0.003</td>
<td>1.771±0.064</td>
<td>1.340±0.009</td>
</tr>
<tr>
<td>Curcumin (15 μM)</td>
<td>2.788±0.271</td>
<td>1.630±0.052</td>
<td>0.917±0.013</td>
</tr>
<tr>
<td>5-fluorouracil</td>
<td>0.528±0.020</td>
<td>0.547±0.040</td>
<td>0.403±0.023</td>
</tr>
<tr>
<td>5-fluorouracil + Curcumin (5 μM)</td>
<td>1.089±0.085</td>
<td>0.596±0.056</td>
<td>0.505±0.051</td>
</tr>
<tr>
<td>5-fluorouracil + Curcumin (15 μM)</td>
<td>1.241±0.090</td>
<td>0.779±0.326</td>
<td>0.642±0.020</td>
</tr>
</tbody>
</table>
Various Incubation Times at 24, 48 and 72h.

Figure 1. Cell Viability was Assessed by MTT Assay in Various Incubation Times at 24, 48 and 72h. treatments using MTT assay shown in Figure 1 and Table 1.

Discussion

Among all types of disorders including chronic disorders [20-22] cancer has furthermore significant. Recently, the effectiveness of various chemotherapeutic on cancer cells were assessed [23-26]. Many chemotherapeutic agents are accompanied with several side effects.

So, in some surveys, herbal agents evaluated in cancer therapy [15, 27-28]. which show acceptable cytotoxic effects.

Our survey evaluates the unique effects of curcumin in combination with 5-FU, to measure how curcumin reduce the cytotoxic effects of 5-Fu in non-Malignant Fibroblast cells- L929.

This survey shows that different doses of curcumin were able to reduce the cytotoxicity of 5-FU on fibroblasts. This is due to this fact that curcumin inhibits the regulation of cell death, and prevents the cytotoxicity of chemotherapeutic agent 5-FU.

In agreement with our results, Haryuna et al indicated that curcumin decreased the Noise-Exposed cochlear fibroblasts death, which was in accordance with our data [29]. In this regards, it could be suggested that curcumin potentially acts as a supportive agent in the prevention and management for fibroblasts damage within chemotherapy of cancer cells.

In a similar research study, curcumin used as an effectual adjuvant to cisplatin cancer treatment. This approach in head and neck cancer could facilitate cisplatin chemoresistance by modifying therapeutic targets and decrease the cisplatin-related ototoxic side effects [30].

An earlier study establishes the impact of curcumin on peroxynitrite-induced damage in rat spiral ganglion neurons. Curcumin revoked cytochrome c release, blocked activation of caspase-3, and changed the expression of the Bcl-2 family [31].

In addition, Curcumin considerably improved nonischemic wound healing in a dose-response versus controls by increased reepithelialization. Enhanced wound healing effects were related to substantial reductions in pro-inflammatory cytokines interleukin (IL)-1 and IL-6. Likewise, Curcumin decreased hypertrophic scarring [32].

In the current survey, with an initial examination of 5-Fu- in non-Malignant Fibroblast cells- L929 cells we examined the impacts of curcumin on the cytotoxicity of 5-Fu by taking different doses.

Overall curcumin as a protective agent against chemotherapy on fibroblast cells exerted the anti-death activity in a dose-dependent manner in all examined times. However, our results, from both in vitro examination, offer the valuable perceptions into the advantages of curcumin on elevating the protective effect and decline the side effects of 5-FU chemotherapy, which is valuable results. However, additional studies proposed to evaluate the effectiveness of curcumin on fibroblast cells.

In conclusion, the current research confirms that curcumin exerted dose-dependent effects even at low concentrations in non-Malignant Fibroblast cells. In other words, all significant effects of curcumin on the viability of the cells were considerable. However, the combined effects of curcumin and 5-Fu showed lower cytotoxic effects in compared to different concentrations of 5-Fu. Curcumin at low concentrations, at some time, can reduce the negative effect of the drug on these cells. Regarding the fact, that combination therapy induced toxicity in L929 cells, it can be concluded that such combination therapy may induce lower toxicity in normal cells and reduce possible side effects.

References

9. Mohammadian M, Zeynali S, Azarbajiani AF, Khadem...


