Comparison of phototoxic effects of hypericin-mediated photodynamic therapy in HT-29 and Caco-2 colon cancer cells

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Abstract: Hypericin (HYP) is a plant-derived photosensitizer. HYP is preferentially taken up by tumor cells. We designed this study to compare HYP-mediated photodynamic therapy (PDT) in HT-29 and Caco-2 colon cell lines. Cells were treated with 0.04, 0.08, and 0.15 µM HYP concentrations and irradiated. The effect of HYP on metabolic profiles, alterations in lactate dehydrogenase (LDH) leakage, and cell cycle progression was investigated for the first time. Changes in glucose consumption, lactate production, and LDH leakage were analyzed. HYP-induced cell death was quantified by double staining (acridine orange/propidium iodide) and alterations in cell cycle regulation were analyzed with flow cytometry (using propidium iodide). LDH leakage and the number of dead cells were elevated, and glucose consumption and lactate production decreased in a dose- and time-dependent manner. PDT resulted in an induction of apoptosis, mostly at the 0.08 µM HYP concentration. Apoptosis and/or necrosis were increased in the 0.15 µM HYP group. The accumulation of cells in the G2/M phase might account for the growth inhibition in HT-29 and Caco-2 cells with 0.08 µM HYP photoactivation. The observed G2/M arrest suggested that HYP may slow down the growth of colon cancer cells by regulating the cell cycle, leading either to growth inhibition or to initiation of apoptotic pathways.

Key words: Hypericin, LDH leakage, metabolic activity, cell cycle arrest, HT-29, Caco-2

1. Introduction
Colorectal cancer is the second most common cancer among men and the third most common cancer among women worldwide. It causes cancer-related deaths every year (American Cancer Society, 2011). Surgical resection is the only curative treatment; therefore, developing novel agents with low toxicity and high efficacy is important (Yang et al., 2012).

Photodynamic therapy (PDT) is an effective therapeutic approach requiring a nontoxic photosensitizer (PS) drug, oxygen (O2), and irradiation with an appropriate wavelength light, resulting in a selective destruction of tumor cells (Barathan et al., 2013). Hypericin (HYP), derived from the plant Hypericum perforatum, has been used in PDT because of its selective accumulation in cancer cells, high-quantum yields, and low cytotoxicity (Barathan et al., 2013). An antiproliferative effect of HYP was reported in several in vitro and in vivo investigations in colon cancer cells. After HYP-mediated PDT, alterations in LDH leakage, cell cycle regulation, and types of cell death were reported in several cancer cell lines (Sačková et al., 2006; Sanovic et al., 2011). On the other hand, there has been no information about the effect of HYP on glucose and lactate metabolism in colon cancer cells, and especially Caco-2 cells.

Lactate dehydrogenase (LDH) is a good biomarker to investigate cell or tissue behavior as it is a cytoplasmic enzyme, which is in equilibrium under normal conditions. The presence of LDH in culture media can be used to predict the physiological state of different types of cell lines. It is known that there is a quantitative correlation between LDH leakage and the number of dead cells; therefore, an increase in LDH leakage can be used as a marker of cytotoxicity (Legrand et al., 1992).

Alterations in cell metabolism are important, especially for cancer cells. A significant increase in glucose consumption was detected for most primary and metastatic cancer cells (Gatenby and Gillies, 2004). In cancer cells, high glucose consumption resulted in increased lactic acid production, which is also a characteristic feature of these cells. It was reported that as a result of elevated glycolysis, lactate production increased in metastatic colorectal cancer cells (Hoiroyde, 1979).

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As a useful target in the management and therapy for cancer, apoptosis was regarded to be an ideal way of cell elimination and a desired type of cell death after using chemopreventive agents. These treatments may result in alterations in the regulation of the cell cycle and in the reduction of cell proliferation in cancer cells (Nihal et al., 1997).

Regarding colon cancer, the antiproliferative activity of HYP was reported only in HT-29 colon adenocarcinoma cells. The aim of this study was to elucidate the mechanism of the antitumorigenic effects of HYP-mediated PDT in HT-29 and Caco-2 cells and to compare the alterations in metabolic pathways, cell cycle regulation, and cell death.

2. Materials and methods

2.1. Culture conditions and PDT procedure

HT-29 (Grade I) and Caco-2 (Grade II) cell lines were obtained from HÜUK, Foot and Mouth Disease Institute, Ankara, Turkey. The cells were routinely maintained in Dulbecco's modified Eagle medium (DMEM) (HyClone Laboratories, Inc., Logan, UT, USA) supplemented with 10% fetal bovine serum (PAA Laboratories, Linz, Austria) at 37 °C and 5% CO₂ in a humidified incubator. The cells were harvested using trypsin/EDTA (0.02% in PBS without Ca²⁺ or Mg²⁺) (Biochrom GmbH, Germany) throughout the study. Passages 5–15 for HT-29 cells and 10–20 for Caco-2 cells were used for analysis. HYP (HPLC grade, AppliChem, Germany) was prepared as a stock solution in DMSO (final concentration less than 0.1%) and diluted to particular working concentrations according to preliminary experiments (Kılıç Süloğlu et al., 2015). The cells were incubated with different concentrations of HYP (0.04, 0.08, and 0.15 µM) in the dark for 24 h. Prior to irradiation, the medium containing hypericin was replaced with fresh hypericin-free DMEM without phenol red. The irradiation device that was used to prepare HYP concentration and the dark control group (incubated with 0.15 µM HYP (without irradiation)) were also included. The irradiation device consisted of twelve L18W/830 fluorescent tubes (Osram, Berlin, Germany) with maximum emission between 530 and 620 nm, which covers HYP maximum absorbance.

2.2. LDH leakage assay

Cells were harvested using DMEM in a 25-cm² flask at a density of 50 × 10⁴ cells/mL. Cytotoxicity induced by HYP photoactivation was assessed by LDH leakage into the culture medium. Therefore, in this assay only media were used, and cells were removed after PDT. Before and after HYP activation, the culture medium was aspirated and centrifuged at 2000 rpm for 5 min in order to obtain a cell-free supernatant. According to Young (1995), the activity of LDH in the medium was determined for each incubation time using a commercially available kit (Spinreact LDH Pyruvate, Kinetic UV DGKC, Sant Esteve De Bas, Spain) in a Shimadzu UV-1700 spectrophotometer at 340 nm. The LDH released from the control group (100%) was used to normalize the data obtained from treatment groups and was expressed as % of the control. The increase in LDH activity for the two incubation times was presented after at least three independent experiments.

2.3. Metabolic activity tests

Cells were harvested using DMEM in a 25-cm² flask at a density of 50 × 10⁴ cells/mL. Before and after HYP activation, the culture medium was aspirated and centrifuged at 2000 rpm for 5 min, and then glucose and lactate concentrations were measured automatically in a YSI 2700 Select Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA) in triplicates. In this assay, only culture medium was used, and cells were removed after PDT. Glucose consumption and lactate production were calculated from the difference between initial and final concentrations (measurements before and after irradiation) and divided by the time passed (16 h or 24 h).

2.4. Quantification of cell death types by acridine orange (AO) and propidium iodide (PI) double staining

Cells were harvested using DMEM in 4-well chamber slides at a density of 50 × 10⁴ cells/mL. HYP-induced cell death in HT-29 and Caco-2 cells was quantified using AO/PI double staining (AO 25 µg/mL, PI 25 µg/mL) in floating and adherent cells and examined under a fluorescence microscope (Olympus IX71, Japan) according to Foglieni et al. (2001). The evaluation was based on nuclear morphology and membrane integrity. The percentages of viable (green nucleus with intact structure), apoptotic (bright green nucleus, showing condensation of chromatin), secondary necrotic (orange nucleus, showing condensation of chromatin), and necrotic cells (orange nucleus with intact structure) were identified as % in at least 200 cells.

2.5. Cell cycle analysis

Changes in cell cycle distribution were analyzed with flow cytometry according to Riccardi and Nicoletti (2006). Cells were harvested using DMEM in a 25-cm² flask at a density of 50 × 10⁴ cells/mL. At 16 and 24 h after HYP activation, both adherent and floating cells were collected and fixed in cold 80% ethanol at 4 °C overnight. Cells were then centrifuged at 1800 rpm for 5 min, washed, and resuspended in PBS. Thirty units of DNase-free...
RNase were added, and then 100 μL of PI (50 μg/mL) was added for a cell suspension of 2 mL. Afterwards, cells were incubated under dark conditions at 37 °C for 1 h and covered until used. The PI fluorescence of the cells was analyzed by flow cytometry (EPICS XLMCL, Beckman Coulter Inc., Brea, CA, USA). MultiCycle by Phoenix Flow Systems (San Diego, CA, USA) was used to deconvolute the cellular DNA content histograms to obtain the % of cells in the G1, S, and G2/M phases of the cell cycle.

2.6. Giemsa staining for cell morphology
Cells were harvested using DMEM in 6-well plates at a density of 30 × 10^4 cells/mL. Cells were stained with Giemsa in order to evaluate the morphology of the cell and the nucleus. Giemsa is a nonionic, neutral stain, which is specific to the phosphate groups of the DNA. After HYP activation, cells were fixed with methanol (only adherent cells were used), stained with Giemsa (PAA, Pasching, Austria), washed with dH2O, and examined under a light microscope (Olympus BX51, Japan) according to Boya et al. (2005).

2.7. Statistical analysis
A statistical analysis was performed using STATISTICA for Windows. Data were expressed as mean ± standard error (SE) and statistical significance was determined at P ≤ 0.05. Data were analyzed using one-way ANOVA with Tukey’s post hoc test. Factorial ANOVA was used to analyze differences depending on both incubation time and HYP concentration.

3. Results
In a previous study (Kılıç Süloğlu et al., 2015), the analysis of HYP content of cells after PDT was shown. The intensity of HYP fluorescence was measured for each HYP concentration as mean and relative fluorescence intensities, which were increased depending on HYP concentration.

No significant differences were observed between control, DMSO, and dark control groups. Therefore, the dark control group (shown as “control” in figures) and the DMSO control group data were used in order to compare the data.

In this study, we aimed to determine the cytotoxic effect of HYP-mediated PDT by a cytoplasmic LDH leakage assay in order to evaluate the damage in plasma membranes that occurred in the HT-29 and Caco-2 cells. According to the measurements of LDH release into the medium, HYP activation induced cell death in a dose-dependent manner, as evidenced by a 16%–83% increase in HT-29 cells and a 18%–81% increase in Caco-2 cells in LDH release (Figure 1). When we compared incubation times, the increase of LDH activity in HYP groups was higher 24 h after PDT for HT-29 cells. On the other hand, except for the 0.08 μM HYP group, the increase of LDH activity was higher in Caco-2 cells at 16 h of incubation.

Glucose uptake and lactate production were compared in the control and HYP treatment groups. Both HT-29 and Caco-2 cells exhibited reduced glucose uptake and lactate production, depending on the HYP concentration (Figure 2). The glucose metabolic activity was decreased in HT-29 cells 24 h after PDT in the 0.04, 0.08, and 0.15 μM HYP groups as 18%, 8%, and 29%, respectively. Lactate production also decreased 24 h after PDT as 8%, 7%, and 24%, depending on the increase of HYP concentration in the HT-29 cells. Metabolic activity in the HYP groups decreased when compared to the control group 16 h after PDT in the Caco-2 cells, as shown in Figure 2. Additionally, glucose consumption and lactate production decreased depending on HYP concentration. Glucose

Figure 1. Influence of HYP-mediated PDT on the membrane integrity of the HT-29 and Caco-2 cells after 16 h and 24 h of incubation.

*: Statistically significant from control group; †: statistically significant from 0.04 μM HYP group; ‡: statistically significant from 0.08 μM HYP group (P ≤ 0.05).
uptake increased by 80% in the 0.08 µM HYP group 24 h after PDT compared to 16 incubation. Lactate production also increased 24 h after PDT at 89%, 72%, and 64% in the 0.04, 0.08, and 0.15 µM HYP groups, respectively.

The alterations in percentage of cell death types between 16 h/24 h after PDT in the 0.04, 0.08, and 0.15 µM HYP groups in HT-29 cells were as follows: apoptotic cells (8%/8.5%, 19.7%/19%, 7.4%/13.2%), secondary necrotic cells (3.9%/1%, 7.2%/4.1%, 9.7%/6%), and necrotic cells (2%/5.6%, 4.3%/9%, 17.9%/22%), as presented in Figure 3. In the Caco-2 cells, the percentages were as follows (16 h/24 h): apoptotic cells (9.2%/11.8%, 13.7%/20.8%, 10.4%/10.1%), secondary necrotic cells (1.7%/1%, 4%/4.3%, 6%/2.9%), and necrotic cells (2.5%/4%, 13.2%/18.7%, 23%/42%). In the Caco-2 cells, 16 h after irradiation necrotic cell % increased comparable to HT-29 cells. However, 24 h after irradiation necrotic cell % decreased. A significant increase in apoptotic morphology was observed only in groups incubated with 0.08 µM HYP concentrations in both cell populations. In Caco-2 cells, apoptotic cell % decreased 24 h after PDT. After 16 h of incubation, necrosis was higher in the 0.15 µM HYP group and remained the main type of cell death for both incubation times. The fluorescent stainings are also presented in Figures 4–7. The induction of apoptosis after PDT was also supported by DAPI staining (Kılıç Süloğlu et al., 2015) and RT-PCR (caspase-3, survivin, Bcl-2, and Bax expressions) (Kılıç Süloğlu et al., 2016).

The effect of HYP on the cell cycle was assessed in the HYP-treated HT-29 and Caco-2 cells using flow cytometry. Figures 8 and 9 present the DNA histograms of HT-29 cells in each cell cycle phase (G1, S, and G2/M), and Figures 10 and 11 present the results for Caco-2 cells. According to the results, G2/M accumulation increased in the 0.04 µM HYP group compared to the control group. Furthermore, cells that accumulated in S phase increased statistically in the 0.15 µM HYP group compared to the 0.04 µM HYP group after 16 h of incubation. As the % of cells in the G2/M phase increased in the 0.08 µM HYP group, the % of cells in the G1 phase decreased after 24 h of incubation.
Figure 3. Distributions of living, apoptotic, secondary necrotic, and necrotic cell % in HT-29 and Caco-2 cells are presented. The cells were treated with HYP (0.04, 0.08, and 0.15 μM HYP), irradiated at 4 J/cm², and harvested 16 h or 24 h after PDT.

Figure 4. The AO/PI staining in HT-29 cells at 16 h incubation from HYP-mediated PDT. A. Control, B. 0.04 μM HYP, C. 0.08 μM HYP, D. 0.15 μM HYP group. Arrow: Apoptotic cells; diamond: secondary necrosis; asterisk: necrosis.
Figure 5. The AO/PI staining in HT-29 cells at 24 h incubation from HYP-mediated PDT. A. Control, B. 0.04 μM HYP, C. 0.08 μM HYP, D. 0.15 μM HYP group. Arrow: Apoptotic cells; diamond: secondary necrosis; asterisk: necrosis.

Figure 6. The AO/PI staining in Caco-2 cells at 16 h incubation from HYP-mediated PDT. A. Control, B. 0.04 μM HYP, C. 0.08 μM HYP, D. 0.15 μM HYP group. Arrow: Apoptotic cells; diamond: secondary necrosis; asterisk: necrosis.
time in the HT-29 cells. On the other hand, Caco-2 cells responded differently. Accumulation in the G2/M phase was significant only in the 0.08 µM HYP group 16 h after PDT compared to the control group.

After HYP activation, the morphological changes in the nuclei and membranes of the cells were examined with Giemsa staining. Depending on HYP concentration, increased nuclear condensation and fragmentation was observed in the HT-29 cells 16 h (Figure 12) and 24 h (Figure 13) after PDT. In the 0.15 µM HYP group, damage to the cell membrane and lysis were observed, especially 24 h after PDT. In the Caco-2 cells, nuclear condensation and fragmentation were elevated mostly in the 0.08 µM HYP group 16 h after PDT (Figure 14). For both incubation times, cell membrane injury was mostly observed in the 0.15 µM HYP group (Figures 14 and 15).

4. Discussion
This study was performed in order to investigate the changes in the metabolic activities of HT-29 and Caco-2 colon cancer cells after HYP-mediated PDT by measuring glucose consumption, lactate production, and LDH activity. HYP concentrations (0.04, 0.08, and 0.15 µM) and incubation times (16 and 24 h) were determined according to previous studies and preliminary examinations. Due to cell membrane damage, loss of intracellular LDH, and LDH release into the culture medium, the increase of LDH leakage points to cell death. The increased activity of LDH is parallel to the rise in dead cell numbers and decrease in glucose consumption (Legrand et al., 1992). Our results were in accordance with Legrand et al.'s findings (1992). After PDT, LDH leakage and the numbers of dead cells were elevated, although glucose consumption was diminished in both HT-29 and Caco-2 cells depending on HYP concentration. Similarly, in another study conducted with EMT6 mouse mammary carcinoma cells, 0.5 μM and 1 μM HYP treatment as well as 1.5 J/cm² irradiation caused dose-dependent increase in LDH leakage (Johnson et al., 1998).

The results presented in this study have provided evidence that HYP photoactivation plays a critical role in inducing glucose uptake alterations. Activation of both cell lines with HYP resulted in lower metabolic activity as measured by glucose consumption and lactate production. According to the results, especially 24 h after PDT in HT-29 cells and 16 h after PDT in Caco-2 cells, glucose consumption decreased in the HYP treatment groups compared to the control groups. Parallel to diminished glucose consumption, lactate production also decreased in HYP-activated groups. It is well known that cancer cells have altered metabolic profiles and display high rates of glucose uptake and glycolysis. Increased glucose transporter-1 (GLUT1) and type 2 hexokinase (HK2) are
Figure 8. DNA histograms in HT-29 cells 16 h after PDT.
Figure 9. DNA histograms in HT-29 cells 24 h after PDT.
Figure 10. DNA histograms in Caco-2 cells 16 h after PDT.
Figure 11. DNA histograms in Caco-2 cells 24 h after PDT.
also important in tumor progression. HT-29 and Caco-2 cells are mutants for the p53 gene, which is responsible for the regulation of HK2 and can affect glucose metabolism (Dang and Semenza, 1999). Additionally, the hypoxia-inducible factor (HIF) regulates processes such as cell proliferation, metabolism, and target genes such as GLUT-1. Hypericin-mediated HIF degradation and HK inhibition were shown in two human tumor cell lines (Karioti and Bilia, 2010). In our study, it is possible that the glucose metabolism might have been influenced after HYP activation due to the changes in GLUT-1 and HK2 enzyme activities in both cell lines.

Figure 12. The morphological change was directly observed with a light microscope after HT-29 cells stained with Giemsa at 16 h incubation from HYP-mediated PDT. A. Control, B. DMSO, C. 0.04 μM HYP, D. 0.08 μM HYP, E. 0.15 μM HYP group. Arrow head: Apoptotic bodies; arrow: cell lysis; asterisk: nuclear condensation.
Kiesslich et al. (2005) showed that glucose deprivation, induced with PDT in vitro, inhibited the production of glycolytic ATP and affected the cellular energy source. Omission of glucose abolishes the energy supply for apoptosis and causes a shift in necrotic cell death under conditions that would normally cause apoptosis. Likewise, our results showed that apoptosis was the dominant cell death type in the 0.08 μM HYP concentration group and, due to deprived glucose, cell death may be shifted to the necrotic type in the 0.15 μM HYP group in the HT-29 and Caco-2 cells. Recently, Kılıç Süloğlu et al. (2015) clarified the mechanisms underlying the effect of HYP-mediated

Figure 13. The morphological change was directly observed with a light microscope after HT-29 cells stained with Giemsa at 24 h incubation from HYP-mediated PDT. A. Control, B. DMSO, C. 0.04 μM HYP, D. 0.08 μM HYP, E. 0.15 μM HYP group. Arrow head: Apoptotic bodies; arrow: cell lysis; asterisk: nuclear condensation.
PDT on antiadhesive properties and cytoskeletal changes in colon cancer. Additionally, they found that the number of floating cells and the apoptotic index can change depending on colon cell line type, HYP concentration, and incubation time in HT-29 and Caco-2 cells.

Cyclooxygenase-2 (COX-2) plays a significant role in colon cancer progression with its antiapoptotic effect. Whereas HT-29 cells have high COX-2 expression, Caco-2 cells have low COX-2 expression level (Lev-Ari et al., 2005). The percentage of apoptotic cells was

**Figure 14.** The morphological change was directly observed with a light microscope after Caco-2 cells stained with Giemsa at 16 h incubation from HYP-mediated PDT. A. Control, B. DMSO, C. 0.04 μM HYP, D. 0.08 μM HYP, E. 0.15 μM HYP group. Arrow head: Apoptotic bodies, arrow: cell lysis, asterisk: nuclear condensation.
greater in Caco-2 cells than in HT-29 cells 16 h after PDT, possibly due to their low COX-2 expression. It was reported that HYP-mediated PDT of human cancer cells lead to upregulation of the inducible COX-2 (Hendrickx et al., 2003). The effect of 0.08 μM HYP on HT-29 and Caco-2 cells showed the highest incidence of apoptosis. These results are in accordance with RT-PCR results for increased caspase-3 activity in HYP treatment groups (Kılıç Süloğlu et al., 2016). Even though slight changes were seen in the percentage of secondary necrosis, they were not statistically significant.

Colorectal cancer development is associated with a number of genetic alterations that allow cells to escape cell-cycle arrest or apoptosis. The mutation of p53 allows

Figure 15. The morphological change was directly observed with a light microscope after Caco-2 cells stained with Giemsa at 24 h incubation from HYP-mediated PDT. A. Control, B. DMSO, C. 0.04 μM HYP, D. 0.08 μM HYP, E. 0.15 μM HYP group. Arrow head: Apoptotic bodies, arrow: cell lysis, asterisk: nuclear condensation.
the growing tumor to evade cell-cycle arrest at G1 and/or G2/M checkpoints and cause cancer progression (Wang et al., 2000).

Two different human colon carcinoma cell lines were selected for this study because they contained combinations of truncated adenomatous polyposis coli, the mutated p53 gene, and the wild-type K-ras oncogene. Previous reports demonstrated that the potential of HYP for inhibiting cell-cycle progression depends on the cancer cell line and PDT conditions (Sačková et al., 2006). In this study, we characterized the cell-cycle arrest in two HYP-treated colon cancer cell lines. HT-29 cells responded 24 h after PDT in the 0.15 µM HYP concentration group with most cells in G2/M arrest. Caco-2 cells had the highest cell % for G2/M arrest 16 h after PDT in the 0.08 µM HYP group. As shown in the present study, HYP selectively blocked cell-cycle progression at the G2/M phase for both cell lines, but at different incubation times.

Agents that either increase or decrease the degree of checkpoint arrest are under development because they affect the rate of cell division. According to DiPaola (2002), efforts to increase G2/M arrest have been associated with enhanced apoptosis. Parallel to the previous studies conducted with HT-29 cells, G2/M was the most sensitive phase of cells for HYP-mediated PDT and results were in accordance with apoptotic increases in the 0.08 µM HYP group. This is the only study investigating the effects of HYP on cell cycle in Caco-2 cells. Similar G2/M arrest was observed in Caco-2 cells, but at an earlier incubation time (16 h after PDT) compared to HT-29 cells (24 h). In Caco-2 cells, the highest apoptosis % was observed in the 0.08 µM HYP group 16 h after PDT, occurring at the same time as G2/M arrest. The arrests in the G2/M phase were found to be associated with the alterations in proteins related to the cell cycle (cyclins, pRb), and they damaged the microtubule network in HYP-treated HeLa cells (Kleban et al., 2008).

According to morphological observations after Giemsa staining, HYP-treated and untreated cells were compared in the HT-29 and Caco-2 cell lines. Results showed that after HYP photoactivation rounded cells, condensed chromosomes, and disappeared nuclear envelopes were observed compared to the intact nuclei in the control cells. These observations were consistent with induced cell-cycle arrest at the G2/M phase (depending on HYP concentration and incubation time) in HT-29 and Caco-2 cells.

In conclusion, this work demonstrates for the first time that HYP-mediated PDT plays a critical role in alterations in the metabolic pathways that result in decreased glucose consumption and lactate production in two colon cancer cell lines, depending on HYP concentration and incubation time. The increase in LDH leakage was directly proportional to HYP concentration, showing the damage to the cell membrane. After PDT with HYP, remarkably different responses were observed in HT-29 and Caco-2 cells, depending on HYP concentration and incubation time. The effective time for HT-29 cells was 24 h after PDT, and 16 h after PDT for Caco-2 cells. The present results suggest that the different gene profiles of these two cells, which have been reported by Kılıç Süloğlu et al. (2016), may contribute to alterations in the cell cycle, metabolic activity, and cell death mechanism responses in HT-29 and Caco-2 cells. According to the cell cycle analysis, G2/M cell cycle arrest started 16 h after PDT in Caco-2 cells, whereas in HT-29 cells G2/M arrest started 24 h after PDT. Because one of the emerging themes for drug discovery is to control cell-cycle phase progression, HYP would be a good photosensitizer for PDT in colon cancer treatment and further investigations are needed.

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