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Curcumin formulated in solid lipid nanoparticles has enhanced efficacy in Hodgkin's lymphoma in mice

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ABSTRACT

Curcumin reduces Hodgkin's lymphoma (HL) cell growth in vitro, but its unfavorable pharmacokinetics highlight the need for novel in vivo delivery systems. Thus, we explored whether formulation of curcumin in solid lipid nanoparticles (SLN-curc) or d-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) nanoparticles (TPGS-curc) could enhance its efficacy in mice. Curcumin formulated in SLN and in TPGS resulted in higher curcumin plasma levels in mice. Compared to vehicle-treated controls, SLN-curc and TPGS-curc reduced HL xenograft growth by 50.5% ($p < 0.02$) and 43.0% ($p < 0.04$), respectively, while curcumin reduced it by 35.8% ($p < 0.05$). In addition, SLN-curc reduced the expression of proteins involved in cell proliferation and apoptosis (XIAP and Mcl-1) in HL tumor extracts. In HL cells in culture, curcumin decreased the expression of relevant anti-inflammatory cytokines (IL-6 and TNF-α) in a concentration-dependent manner. Moreover, when given in combination with bleomycin, doxorubicin and vinblastine, curcumin showed an additive growth inhibitory effect. In conclusion, SLNs appear as an appropriate and effective drug delivery system for curcumin. Given the efficacy of SLN-curc and the enhanced growth inhibitory effect when combined with chemotherapeutic drugs, we speculate that curcumin, when appropriately formulated, is a promising adjuvant agent for the treatment of HL and merits further evaluation.

1. Introduction

Hodgkin's lymphoma (HL) is a significant health problem. The malignant component of the tumor, the large mononuclear Hodgkin and the multinuclear Reed-Sternberg (H-RS) cells, often constitutes only 1–2% of the total tumor mass in the lymph nodes. The bulk of the tumor contains non-neoplastic inflammatory cells. Despite successful advances in the treatment of HL with multi-drug approaches, its toxicity becomes evident after several years as secondary malignancies and cardiovascular diseases [1]. Therefore, alternative therapies that specifically target deregulated signaling cascades critical for HL growth are urgently needed.

Curcumin (diferuloylmethane) is a major bioactive component of turmeric, a widely used natural food product in curry powder and food coloring (mustard) [2]. Curcumin is a very unique natural compound due to two contradictive issues: it is a potential therapeutic agent for a wide range of human diseases [3], but problematic in terms of formulation and delivery development. We have previously shown that curcumin reduces HL cell growth [4]. In H-RS cells in culture, we observed that curcumin: *1)* inhibited the activation of STAT3 and NF-κB, two critical molecular contributors to HL cell growth; *2)* decreased the expression of proteins involved in cell proliferation and apoptosis; *3)* blocked cell cycle progression; and *4)* triggered cell death by apoptosis; all of these contribute to the significant reduction (80–97%) in H-RS cell growth by curcumin [4]. Our findings provide a mechanistic rationale for the potential use of curcumin in vivo. However, curcumin's low water solubility, poor in vivo bioavailability and unfavorable pharmacokinetic profile limit its efficacy as anti-cancer agent in vivo. Thus, approaches to enhance curcumin's bioavailability for its potential use in vivo are clearly needed.

Over the past decade, there has been an increasing interest in using nanotechnology for cancer therapy, in order to enhance drug efficacy and lower drug toxicity. Among these, the mode of drug delivery is an important determinant of in vivo drug efficacy. Nanocarriers are emerging tools that have demonstrated great potential in the delivery of lipophilic drugs by: *1)* enhancing the bioavailability of the drugs; *2)* aiding in the dissolution of drugs in aqueous solution above their solubility limit; *3)* stabilizing the drug; *4)* specific delivering to target site (s); and *5)* overcoming multidrug resistance [5]. Among various nanocarriers, solid lipid nanoparticles (SLNs) are being increasingly

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appreciated as alternative drug carrier systems to traditional polymeric nanoparticles [6]. SLNs consist of a solid lipid core, in which lipophilic therapeutics, such as curcumin, can be efficiently entrapped and released in a controlled manner. SLNs have been reported to efficiently improve pharmacokinetic profile [7,8], targeted drug delivery to tumor sites and efficacy, while minimizing systemic side effects [9,10]. For example, we have shown that incorporating the novel agent phosphosulindac in SLNs enhances its efficacy against human lung cancer in preclinical models by enhancing the pharmacokinetics profile of PS and leading to enhanced delivery of PS to the cancer tissue [11]. Moreover, multiple investigators have successfully evaluated the use of SLN to formulate curcumin in various disease models [12,13].

Besides SLNs, formulation of curcumin in d-α-Tocopheryl polyethylene glycol 1000 succinate-stabilized curcumin (TPGS-curc) has also been extensively study [14,15]. TPGS-curc provides a better in vivo kinetic profile than curcumin. Besides its use in formulating curcumin, a formulation containing paclitaxel formulated in TPGS has shown improved oral bioavailability in rats by about 6-fold [16]. Therefore, SLN and TPGS appear ideal formulations to enhance curcumin's bioavailability in vivo.

In the current study, we assessed whether formulating curcumin in SLN (SLN-curc) and in TPGS (TPGS-curc) could improve the pharmacokinetics of curcumin and enhance its anticancer effect in an animal model of HL. In addition, we explored whether curcumin could enhance the cell growth inhibitory effect of current chemotherapeutic drugs [17,18]. We show that primarily SLN-curc, and to a lesser extent TPGS-curc can enhance curcumin plasma levels and lead to a superior anticancer growth inhibition, compared to curcumin alone. Moreover, curcumin shows an additive effect in inhibiting HL cell growth when given in combination with chemotherapeutic drugs. Our results further support the anticancer potential of curcumin and support the use of SLN for its delivery.

2. Materials and methods

2.1. Reagents

Curcumin (> 97% purity) was purchased from Thermo Fisher Scientific (Waltham, MA). We prepared a 100 mM stock solution of curcumin in DMSO. Doxorubicin and Vinblastine were purchased from Tocris (Minneapolis, MN, USA), whereas Bleomycin was purchased from Tokyo Chemical Industry Co., Ltd (Portland, OR, USA). Stearic acid, lecithin, chloroform, Myrj59 and TPGS was purchased from Millipore-Sigma (St Louis, MO, USA). All general solvents and reagents were of HPLC grade or the highest grade commercially available.

2.2. Cell culture

Hodgkin lymphoma L-540 cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). We have not authenticated this cell line, however we routinely test for mycoplasma contamination in every cell line every three months. These cells were grown as monolayers in the specific medium and under conditions suggested by DSMZ. All the cell lines were characterized by cell morphology and growth rate and passaged in our laboratory less than 6 months after being received. Since curcumin was resuspended in dimethylsulfoxide (DMSO), controls for cells incubated only with DMSO were run for each condition [maximum concentration of DMSO in the media was 0.25% (v/v)].

2.3. Cell viability assay

Following the treatment with various concentrations of curcumin alone or either bleomycin, vinblastine or doxorubicin for 48 and 72 h, cell growth was determined by CellTiter-Glo Luminescent cell viability assay (Promega, Madison, WI, USA). Briefly, L-540 cells (10,000 cells in 100 µl media) were incubated in the absence or presence of 5–20 µM curcumin, bleomycin, vinblastine or doxorubicin alone or in combination for different time periods. Cell growth was determined following the manufacture's protocol (Promega, Madison, WI, USA).

2.4. IL-6 and TNF-α levels

The levels of IL-6 and TNF-α cytokines were measured in L540 cell culture media using ELISA assays (R&D Systems, Minneapolis, MN, USA) following the manufacturers' protocols. Briefly, 1.5×10^6 cells were pre-incubated with curcumin (10, 20 and 40 µM) for 60 min followed by lipopolysaccharide (LPS) 100 ng/ml treatment for 5 h in under the indicated experimental conditions. After the incubation, the cell culture media were collected and centrifuged at 800 × g for 10 min. We determined the various cytokines in the supernatant by ELISA following the manufacturers' protocols.

2.5. Preparation and characterization of curcumin formulations

2.5.1. SLN-curcumin preparation

SLN-curc was prepared using an emulsion/evaporation technique as previously published [11]. Briefly, 450 mg Myrj59 was dissolved in 60 ml deionized water. The aqueous solution was heated to 75 °C in a water bath under constant stirring at 700 rpm. The organic phase consisted of 380 mg stearic acid, 200 mg lecithin and 300 mg curcumin dissolved in 20 ml chloroform. The organic phase was gently injected into the aqueous phase with a syringe. The speed of the stirrer was then increased to 1000 rpm. After 2.5 h (when the volume of the emulsion was reduced to about 5–10 mL), 10 mL of ice-cold water was added and stirring continued for another 2 h at 1000 rpm. The resulting suspension was centrifuged at 7,200 × g for 1 h and the supernatant was discarded. The pellet was washed twice by re-suspending in deionized water followed by centrifugation to remove the supernatant. The final pellet was re-suspended in deionized water, frozen at −20 °C overnight, and freeze-dried.

2.5.2. TPGS-curcumin preparation

50 mg of TPGS and 3 mg of curcumin were dissolved in 5 ml chloroform in a glass flask. Once dissolved, chloroform was evaporated at 40 °C until a thin film was formed. Approximately 1 h later, 2 ml of PBS were added, vortexed and then bath sonicated for 15 min.

2.5.3. Characterization

The size and ζ (zeta) potential of the nanoparticles were determined using dynamic light scattering (DLS) and microelectrophoresis, respectively, 10 min after diluting the samples in PBS and housing them at 25 °C using a Zeta-Plus Brookhaven instrument (Holtsville, NY). Entrapped curcumin was quantified with high-performance-liquid-chromatography (HPLC) Waters Alliance 2695 equipped with a Waters 2998 photodiode array detector (220 nm) (Milford, MA) and a Hypersil C18 column (150 × 4,6 mm, particle size 3 µm; Thermo Scientific, Waltham, MA). We dissolved a small known amount of lyophilized nanoparticle suspension into 1 ml of acetonitrile to determine the drug loading and encapsulation efficiency for the formulation. The mobile phase followed a gradient between buffer A (H_2O, acetonitrile, trifluoroacetic acid 94.9:5:0.1 v/v/v) and buffer B (acetonitrile).

2.6. Western blot analysis

Total tumor fractions were obtained as previously described [4]. Aliquots of total fractions containing 25–80 µg protein were separated by reducing 7.5–12.5% (w/v) polyacrylamide gel electrophoresis and electroblotted to Polyvinylidene fluoride (PVDF) membranes. The membranes were probed overnight with antibodies against XIAP (Cat

number # 14334), survivin (Cat number # 2808), Bcl-xL (Cat number # 2764), Mcl-1 (Cat number # 4572), Bcl-2 (Cat number # 15071) or p21 (Cat number # 2946), all from Cell Signaling Technologies (Danvers, MA, USA) antibodies (1:1000 dilution). β-actin (Cat number # A1978; Sigma-Aldrich) was used as the loading control. Following incubation, for 90 min at room temperature, in the presence of the corresponding secondary antibody (HRP-conjugated; 1:5000 dilution), the conjugates were visualized by chemiluminescence.

2.7. Animal studies

All animal studies were approved by the Institutional Animal Care and Use Committee at Stony Brook University.

2.7.1. Pharmacokinetics

BALB/c female mice 5–6 week old (Taconic, Hudson, NY) were administered with curcumin, SLN-curc or TPGS-curc given once orally as a single dose at 100 mg/kg of curcumin and euthanized at different time points (0.5, 1, 2 and 4 h). Blood samples were collected by cardiac puncture. Plasma was extracted with acetonitrile and curcumin plasma levels were determined by HPLC. Pharmacokinetic parameters in plasma determined include maximal plasma concentration (C_{max}), time to reach maximal plasma concentration (T_{max}) and the area under the concentration–time curve (AUC).

2.7.2. Efficacy study in nude mouse xenografts

Female SCID mice at 6 weeks of age were purchased from Charles Rivers (Wilmington, MA), and maintained in pathogen-free conditions with irradiated chow. Animals were bilaterally, s.c. injected with 5×10^6 L-540 cells/tumor in 0.1 ml sterile PBS/Matrigel (ratio 1:1). When L-540 cells reached palpable tumors, mice (n = 8/group) were divided randomly into three groups receiving control, curcumin, SLN-curc or TPGS-curc (100 mg/kg of curcumin), given orally to mice once daily, five times per week during 18 days. All agents were given equal concentrations of curcumin content as determined by HPLC. Body weight was measured once a week while tumors were measured twice weekly. Tumor sizes were calculated by the formula: [length x width x (length + width/2) x 0.56] in cubic millimeters. At the end of the experiments, animals were euthanized by CO_2 asphyxiation. Blood and tumor tissue were collected for drug levels and for immunoblots.

2.8. Statistical analysis

The data, obtained from at least three independent experiments, were expressed as the *mean ± SEM*. Statistical evaluation was performed by one-factor analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons. $P < 0.05$ was regarded as being statistically significant.

3. Results

3.1. Characterization of SLN-curcumin and TPGS-curcumin formulations

SLN-curc had a mean diameter of 125.2 nm with their polydispersity index being 0.268 ± 0.005 and a zeta potential value of −19.4 ± 2.2 mV. On the other hand, the mean particle size for TPGS-curc was 285 nm, the zeta potential was −21.2 ± 2.6 mV, and the polydispersity index was 0.283 ± 0.006. Importantly, both SLN-curc and TPGS-curc increased the level of curcumin in the plasma. For instance, the AUC_{0-4h} for SLN-curc, TPGS-curc and curcumin alone was 1,508, 1042 and 231.5 ng/ml × h, respectively (Fig. 1), indicating that curcumin when formulated in SLN presents a better pharmacokinetic profile than TPGS-curc and curcumin alone.

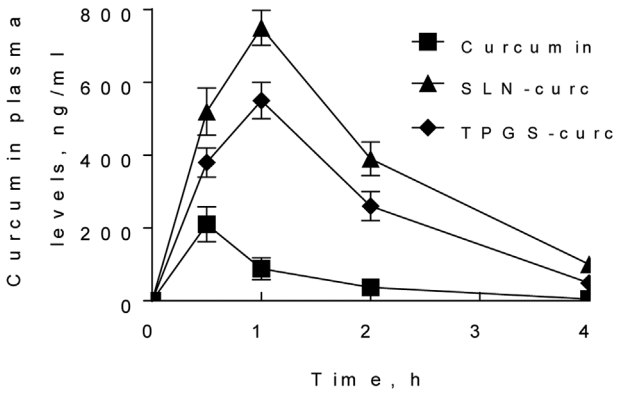


Fig. 1. Formulation of curcumin in SLN and TPGS increases curcumin plasma levels. The pharmacokinetics of curcumin administered orally by gavage to mice. Curcumin, curcumin formulated in SLN (SLN-curc) or curcumin formulated in TPGS (TPGS-curc) (100 mg/kg of curcumin) was administered to mice as a single dose orally. Plasma levels of curcumin were determined by HPLC at the times shown.

3.2. Effect of curcumin, SLN-curcumin and TPGS-curcumin on the growth of heterotopic human Hodgkin's lymphoma xenografts

To assess the chemotherapeutic potential of curcumin in vivo, we used a Hodgkin's lymphoma heterotopic xenograft model. Subcutaneously injected L-540 human Hodgkin's lymphoma cancer cells gave rise to exponentially growing tumors in SCID mice. Once the L-540 tumors reached ~450 mm^3, the mice were randomized into the various treatment groups. All the treatment groups were treated with curcumin at a dose of 100 mg/kg/d, 5 days per week. This was based on our in vitro HPLC curcumin determinations. As shown in Fig. 2A, curcumin, significantly inhibited L-540 xenograft growth, starting on day 15 of treatment until the end of the study ($p < 0.05$, vs. control). Formulation of curcumin in SLN and TPGS enhanced its inhibitory effect. Compared to control, SLN-curc and TPGS-curc reduced the rate of growth over baseline, by 50.5% ($p < 0.02$) and 43.0% ($p < 0.04$), respectively (Fig. 2A). To note, there was no significant differences between the tumor-growth curves of curcumin and TPGS-curc ($p = 0.30$), as well as curcumin and SLN-curc groups ($p = 0.13$). Regarding their safety, curcumin, SLN-curc and TPGS-curc were well tolerated, with the mice showing no weight loss or other signs of toxicity during treatment (Fig. 2B).

At euthanasia, we also determined the levels of curcumin in the tumor lysates. As shown in Fig. 2C, curcumin levels were detected in the tumor tissues treated with curcumin, SLN-curc and TPGS-curc. When compared to curcumin group, SLN-curc and TPGS-curc resulted in a 4.5- and 3.0-fold increase in tumor curcumin levels, respectively ($p < 0.05$; Fig. 2C). Interestingly, we observed an inverse correlation between tumor growth and curcumin levels in the tumor lysates (Fig. 2D).

3.3. Regulation of anti-apoptotic markers by curcumin, SLN-curcumin and TPGS-curcumin in HL xenografts

We have previously shown that curcumin down-regulates NF-κB- and STAT3-dependent gene expression in HL cells, including XIAP, survivin and Bcl-xL [4]. Thus, we now explored whether curcumin, SLN-curc and TPGS-curc could inhibit NF-κB- and STAT3-dependent gene expression in vivo, evaluating the levels of XIAP, Bcl-xL, survivin, Mcl-1, Bcl-2 and p21 in L-540 tumor lysates.

Of the anti-apoptotic markers, XIAP levels were reduced by all three experimental groups. In comparison to the control group, curcumin down-regulated XIAP by 19% ($p = 0.0043$), while TPGS-curc did so by 47% ($p < 0.001$; Fig. 3A). However, SLN-curc had the greatest effect

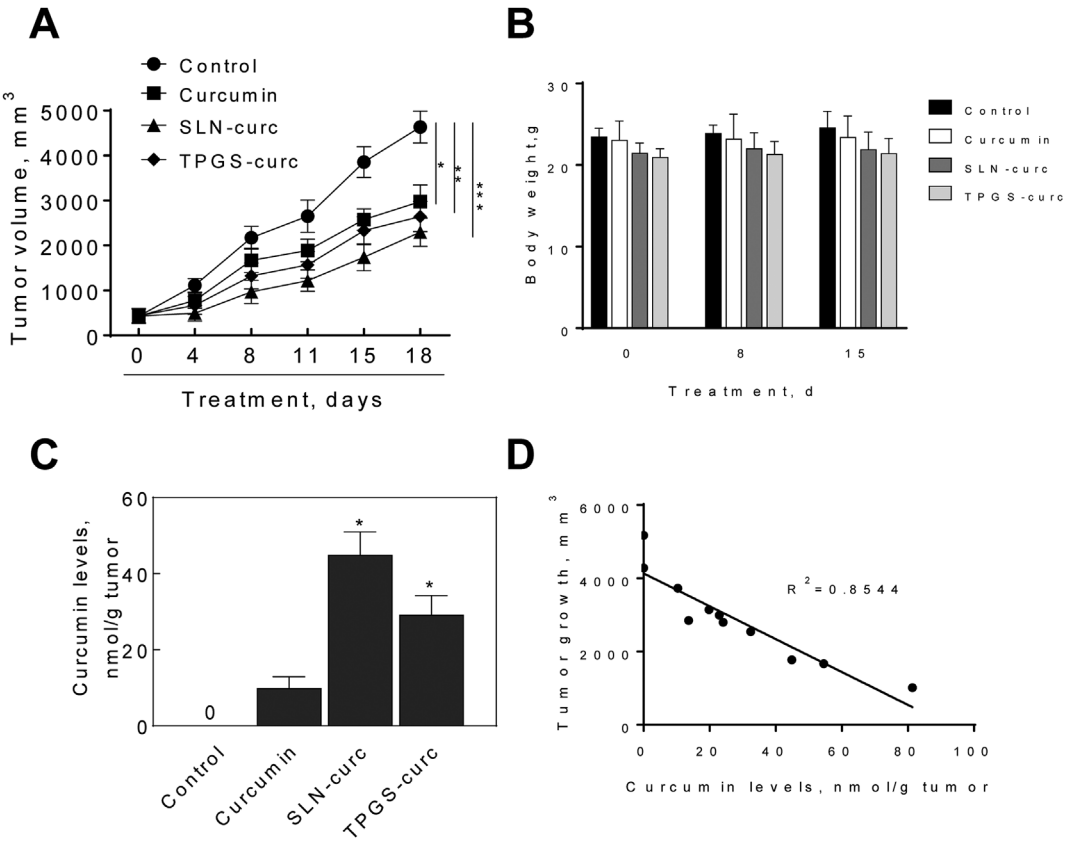


Fig. 2. Curcumin and curcumin formulated in SLN or TPGS inhibit the growth of heterotopic human Hodgkin's lymphoma xenografts. (A) Effect of curcumin and curcumin formulated in SLN (SLN-curc) or TPGS (TPGS-curc) on the growth of L-540 xenografts. SCID mice bearing L-540 xenografts (n = 8/group) were treated with curcumin, or various curcumin formulations given by oral gavage at a dose of 100 mg/kg (of curcumin), 5×/week for 18 days. L-540 tumor volume growth over time is shown. Curcumin, TPGS-curc and SLN-curc groups show significant reduction in tumor size compared to control group [*Control vs. curcumin ($p < 0.05$); **Control vs. TPGS-curc ($p < 0.04$); ***Control vs. SLN-curc ($p < 0.02$)]. **(B)** Mice body weight over time for control, curcumin, SLN-curc and TPGS-curc. Results are expressed as the mean ± SEM. **(C)** Curcumin levels in tumors. Curcumin levels were determined by HPLC. *Significantly different compared with curcumin group; $P < 0.05$. **(D)** Inverse correlation between antitumor effects with tumor curcumin level.

reducing XIAP levels by 54% ($p < 0.001$). No significant changes were noted between SLN-curc and TPGS-curc groups, though. The levels of survivin, Bcl-2 and Bcl-xL were not significantly changed among the various experimental groups (Fig. 3A–B). On the other hand, Mcl-1 levels expression was reduced by 44% ($p = 0.0005$) only by the SLN-curc group (Fig. 3B). Both curcumin and TPGS-curc showed no significant changes in Mcl-1 expression compared to the control group (Fig. 3B).

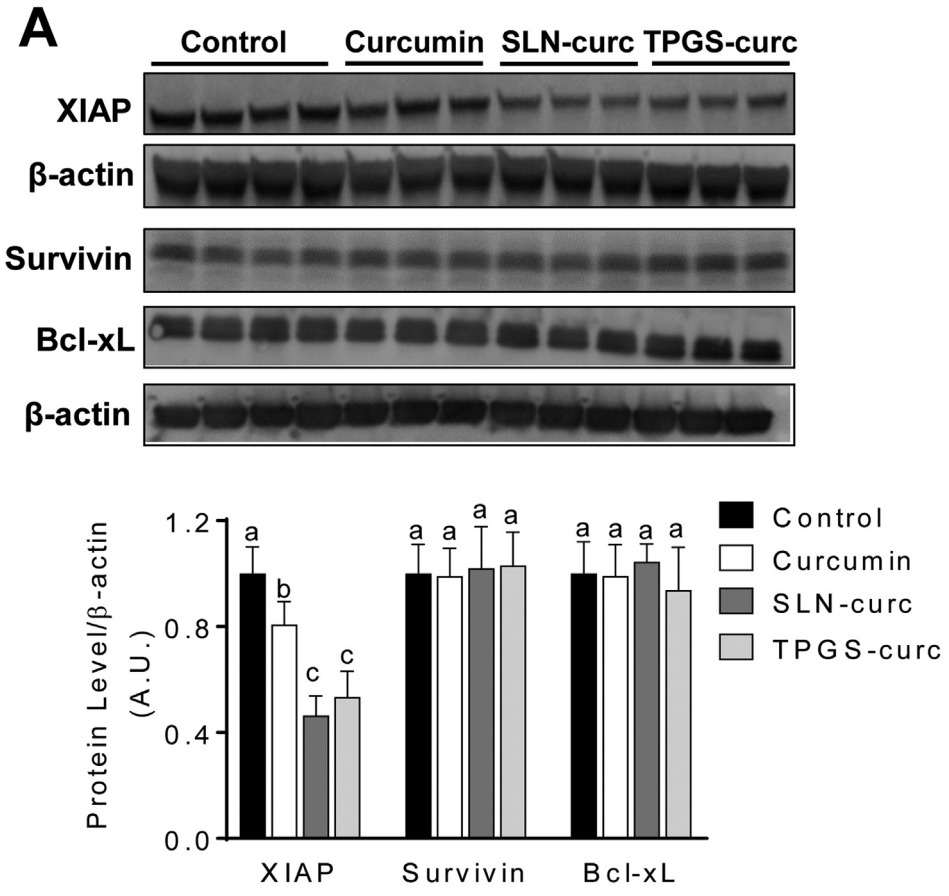
We subsequently explored whether curcumin, SLN-curc and TPGS-curc could regulate cell growth by affecting the cell cycle, by determining the expression of p21 in tumor lysates. As shown in Fig. 3B, SLN-curc increased p21 levels by 29% ($p = 0.0121$). However, the levels of p21 in curcumin and TPGS-curc groups were similar to those in the control group (Fig. 3B).

3.4. Curcumin reduces IL-6 and TNF-α release in L-50 cells

Survival and proliferation of HL cells are influenced by many cytokines produced by different cell types in the lymph node microenvironment. We have previously shown that in HL cells curcumin inhibited the activation of NF-κB and STAT3 [4], two master regulators of inflammation. Thus, we now determined whether this inhibition of inflammatory signaling corresponded to reduced pro-inflammatory cytokine secretion, by investigating whether curcumin affects the production of TNF-α and IL-6 in L-540 cells. As the baseline levels of TNF-α and IL-6 were very low or undetectable, we stimulated these cells with LPS. L-540 cells were pre-incubated for 1 h with various concentrations of curcumin followed by treatment with LPS for an additional 5 h and the response of these two cytokines was determined. In L-540 cells, LPS induced a brisk production of both TNF-α and IL-6 levels. Curcumin at 20 and 40 μM prevented the increase in the expression of the pro-inflammatory cytokines TNF-α, (by 55% and 69%, respectively) and IL-6 (by 42% and 62%, respectively) in L-540 cells (Fig. 4).

3.5. Curcumin enhances the growth inhibitory effect of chemotherapeutic drugs in L-540 cells

Growing preclinical and clinical evidence indicates that combination therapies increase the anticancer efficacy without increasing toxicity [19]. Bleomycin, doxorubicin and vinblastine are three chemotherapeutic drugs currently being used for HL treatment in the clinic [17,18]. Therefore, we evaluated in L-540 cells whether curcumin could enhance the growth inhibitory effect of these drugs. Curcumin alone led to a concentration- and time-dependent reduction of L-540 cell growth (Fig. 5A). Treatment of L-540 cells with bleomycin, doxorubicin and vinblastine for 48 h resulted in a concentration-dependent decrease in cell viability. When combined with curcumin 5 μM, there was an additive effect. For example, treatment of L-540 cells for 48 h with 2 mg/ml bleomycin and 5 μM curcumin, when given alone, led to a 21% and 44% reduction in cell growth, while together they resulted in a 66% reduction in L-540 cell growth (Fig. 5B). A stronger additive effect was observed for doxorubicin, where the combination of curcumin 5 μM plus doxorubicin 0.4 mg/ml reduced cell growth by 79%, whereas each drug alone reduced L-540 cell growth by 44% and 23%, respectively



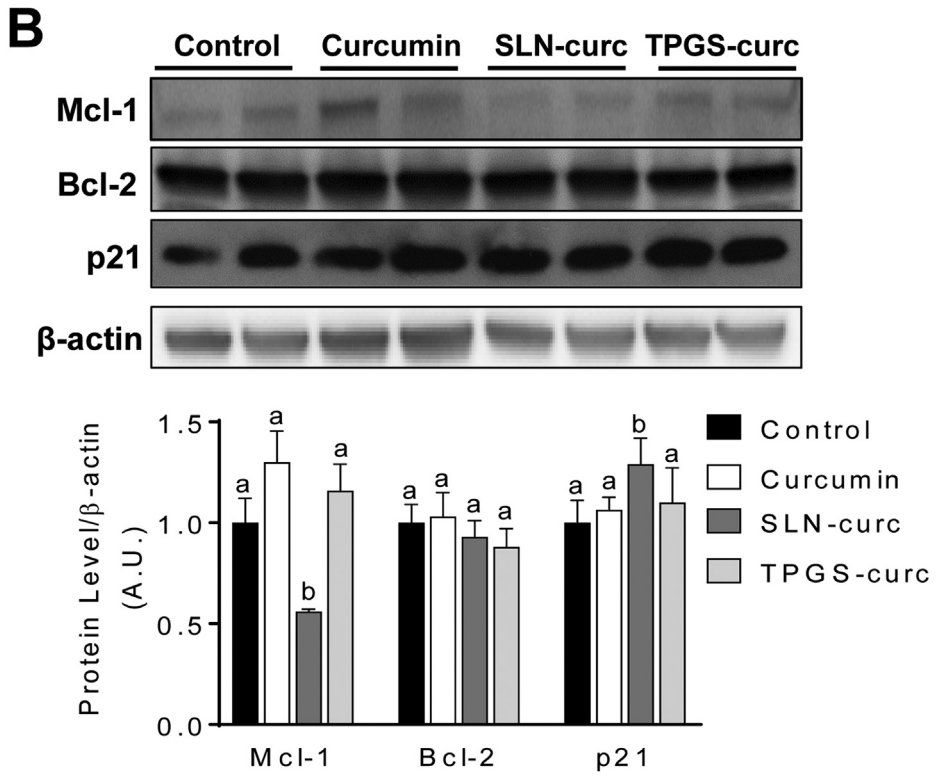


Fig. 3. Effect of curcumin formulated in SLN or TPGS on select anti-apoptotic and cell proliferation markers. (A) L-540 xenograft tumor lysates were analyzed for XIAP, Bcl-xL and survivin by immunoblotting. Loading control: β-actin. Each lane represents a different tumor sample. Bands were quantified and results are expressed as the ratio of total expression levels over β -actin for each protein. Different letters indicate significant differences ($p < 0.05$) between groups; whereas the same letter indicating no significant differences in protein expression levels. All protein levels are expressed as a ratio to β-actin in A.U. and normalized to the control group. **(B)** L-540 xenograft tumor lysates were analyzed for Mcl-1, Bcl-2 and p21 expression levels by immunoblotting. Loading control: β-actin. Each lane represents a different tumor sample. Bands were quantified and results are expressed as the ratio of total expression levels over β -actin for each protein. Different letters indicate significant differences ($p < 0.05$) between groups; whereas the same letter indicating no significant differences in protein expression levels. All protein levels are expressed as a ratio to β-actin in A.U. and normalized to the control group.

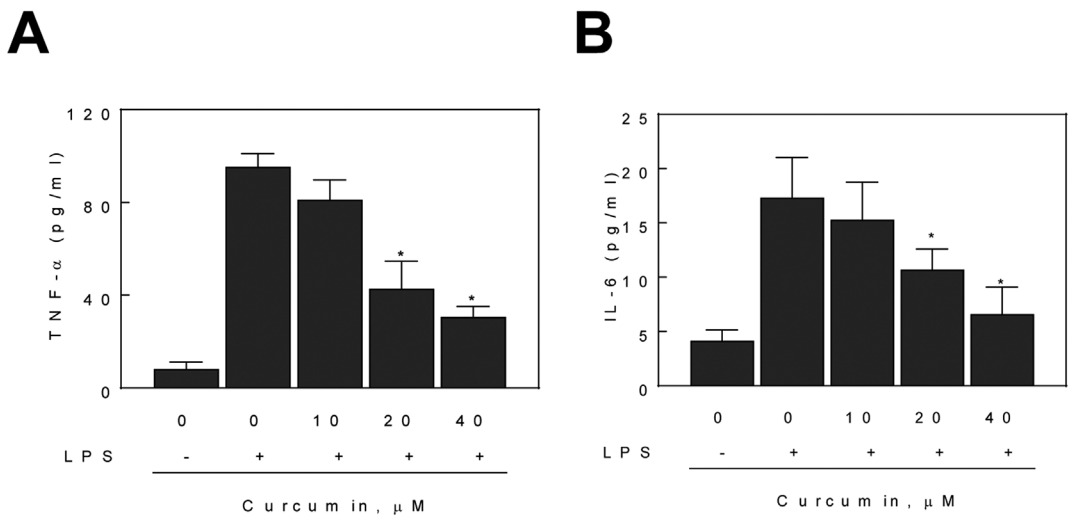


Fig. 4. Curcumin reduces the levels of inflammatory cytokines. L-540 cells were pre-treated for 1 h with curcumin (10–40 μM) followed by treatment with lipopolysaccharide (LPS; 100 ng mL^{-1}) for an additional 5 h. The concentration of tumor necrosis factor-α (TNF-α) **(A)** and IL-6 **(B)** in culture media was measured by ELISA. Values are mean ± SEM of three independent experiments. *Significantly different compared with LPS-stimulated group; $P < 0.05$.

(Fig. 5C). In L-540 cells the cytotoxic effect of curcumin and vinblastine was only additive (Fig. 5D). While 1 mg/ml vinblastine or 5 μM curcumin at 48 h reduced cell growth by 45% and 44%, respectively, together they resulted in a 81% reduction in L-540 cell growth. These results suggest that curcumin could be a useful combination partner of current chemotherapeutic drugs in the treatment of HL.

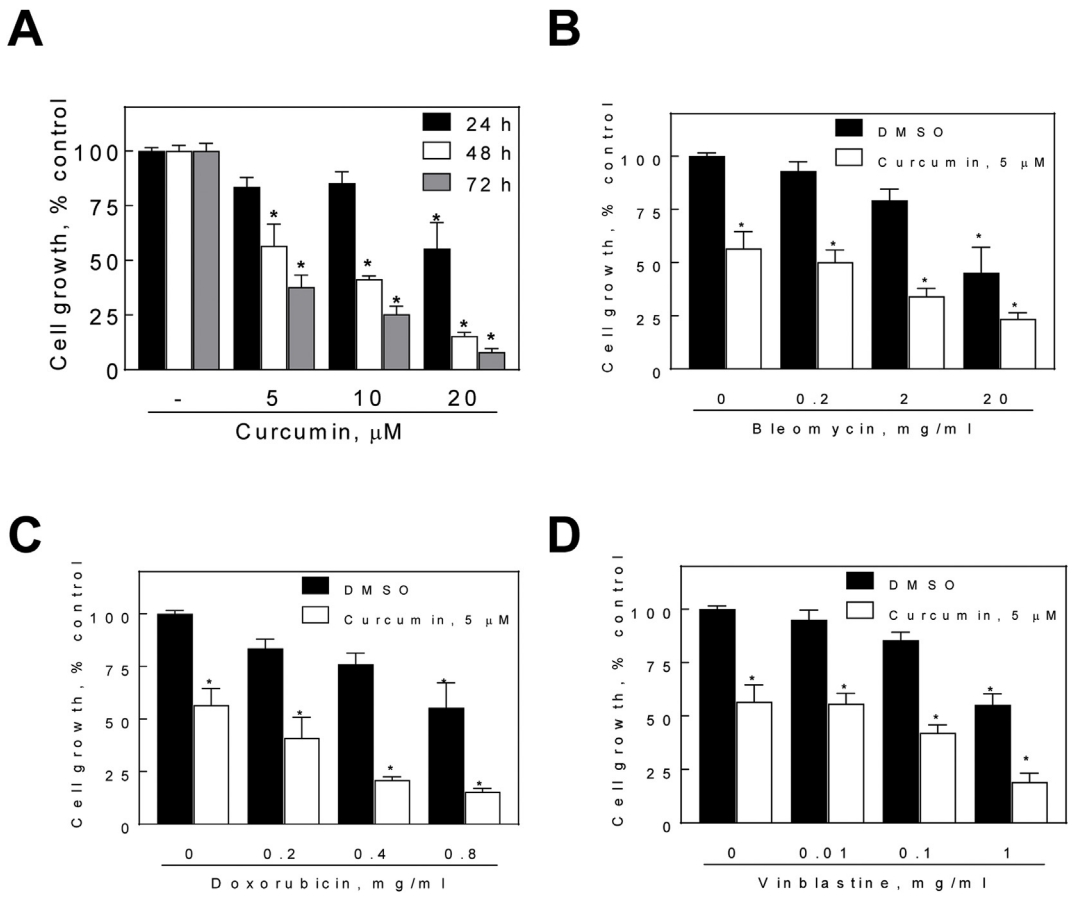


Fig. 5. Curcumin enhances the growth inhibitory effect of bleomycin, doxorubicin and vinblastine in L-540 cells. (A) Curcumin reduces L-540 cell growth in a time- and concentration-dependent manner. Cell viability was determined in L-540 cells after 24, 48 and 72 h of incubation with curcumin (5–20 μM). Results are expressed as percentage of control. *Significantly different compared with control group; $P < 0.05$. **(B–D)** Curcumin enhances the growth inhibitory effect of bleomycin (B), doxorubicin (C) and vinblastine (D) in L-540 cells. Cell viability was determined in L-540 cells after 48 h of incubation with various concentrations of the chemotherapeutic drugs alone (DMSO) or in combination with curcumin (5 μM). Results are expressed as percentage of control. *Significantly different compared with DMSO-treated groups; $P < 0.05$.

4. Discussion

The current therapeutic strategies for HL are to retain efficacy and minimize long-term toxicity. Curcumin is considered as a safe compound that has function on inflammatory and cancer treatments. However, despite its demonstrated effects, the potential health benefits of curcumin are limited by its poor absorption, rapid metabolism and rapid systemic elimination. Our work establishes that incorporating curcumin in SLN and in TPGS enhances its efficacy against HL in preclinical models. The superior efficacy of SLN-curc and to a lesser extent of TPGS-curc in vivo is due to an improvement of pharmacokinetic parameters leading to enhanced delivery of curcumin to the cancer tissue.

Curcumin has been extensively evaluated for its potential use in cancer treatment [20,21]. However, the application of curcumin in vivo is challenged by its rapid half-time and poor bioavailability. These reasons led us to explore ways to improve curcumin's bioavailability. Curcumin has been loaded into several carriers, such as lipids, in order to enhance the drug stability, largely increase the plasma concentration and significantly improve the efficiency [22]. In our study, we chose SLNs and TPGS as carriers for curcumin. The pharmacokinetics profile of SLN-curc and TPGS-curc on mice was increased compared to plain curcumin, indicating that these were appropriate carriers for curcumin.

The efficacy of SLN-curc was stronger than that of TPGS-curc and curcumin alone, and even though these effects only trended toward statistical significance (particularly in the case of SLN-curc group), such lack of significance is probably due to lack of power. Nonetheless, we deem this finding as highly relevant. An important finding was the detection of curcumin in the tumor which was higher in the SLN-curc group, and the fact that the tumor levels inversely correlated to tumor volume. These findings suggest that enhanced efficacy of SLN-curc may be closely associated with the improved delivery of curcumin to the L-540 tumors. Importantly, the dose used for SLN-curc and TPGS-curc seems to be safe, given that the body weight of SLN-curc and TPGS-curc treated mice throughout the treatment remained equivalent to the control group and no other adverse reaction was observed. The reduction in tumor growth was associated with the modulation of anti-proliferative and pro-apoptotic genes by curcumin, which include STAT3 and NF-κB related genes [4]. For instance, SLN-curcumin significantly reduced XIAP and Mcl-1 levels, compared to control, as well as increasing the levels of p21, a critical cell cycle regulator.

IL-6 is a potent immunomodulatory cytokine that has pathogenic and prognostic significance in a number of disorders [23]. Previous studies in HL have demonstrated the association between elevated serum levels of IL-6 and unfavorable prognosis, including advanced stage and with reduced survival [24]. Similarly, the plasma levels of TNFα and its soluble receptors have also been shown to be higher in HL patients and to correlate with clinical features and outcomes. In L-540 cells, curcumin significantly reduced the expression of both of these cytokines in a concentration-dependent manner. Given that HL cells might support tumor survival and expansion by producing cytokines [25], our findings raise the possibility that curcumin could also be beneficial by reducing cytokine production. However, these results should be taken carefully since they were obtained in HL cells in culture and confirmation with HL patient's samples is warranted.

The current standard of care for HL patients include the use of drugs in combination [26–30]. Contingent on the clinical stage and the associated risk factors, around 80% of the HL patients can be cured with conventional treatment, such as the standard ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) chemotherapy regimen, which results in a high cure rate with acceptable toxicity [26–30]. However, up to 30% of patients with advanced HL will still progress or relapse. Recent studies have focused on the development of novel potential therapeutic agents for HL. For this reason, we explored the potential benefit of curcumin to current chemotherapeutic drugs.

Indeed, combining agents for the treatment of cancer is a promising area of investigation [31]. The possible favorable outcomes for drug combinations include: 1) increasing the efficacy of the therapeutic effect; 2) avoiding toxicity by decreasing drug dosage while increasing or maintaining the same efficacy; and 3) minimizing the development of drug resistance. Because of these benefits, drug combinations are being widely used and are an excellent choice for the treatment of cancer [31]. Interestingly, curcumin is a successful combination partner of doxorubicin, vinblastine and bleomycin, enhancing the growth inhibitory effect of these drugs. The anti-proliferative effect of curcumin when combined with bleomycin, doxorubicin or vinblastine in the L-540 cells far exceeded that of the chemotherapeutic drugs alone. Although these results cannot be extrapolated to animals or humans, they raise the possibility that curcumin could be a successful adjuvant drug for HL treatment. We therefore believe that curcumin is a promising adjuvant agent for the treatment of HL and may also serve as a chemosensitizer to improve the therapeutic efficacy of other anticancer drugs.

In summary, curcumin is an effective anticancer agent in preclinical models of HL, and a potential adjuvant drug for therapy. Furthermore, its formulation in SLN nanoparticles improves its pharmacokinetics and enhances its efficacy. Our data further indicate curcumin as a candidate agent for HL and suggest its formulation in SLN for its administration.

Conflicts of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.abb.2018.04.012.

References

[1] D. Re, R.K. Thomas, K. Behringer, V. Diehl, From Hodgkin disease to Hodgkin lymphoma: biologic insights and therapeutic potential, Blood 105 (12) (2005) 4553–4560.
[2] A. Goel, A.B. Kunnumakkara, B.B. Aggarwal, Curcumin as “Curecumin”: from kitchen to clinic, Biochem. Pharmacol. 75 (4) (2008) 787–809.
[3] A. Goel, S. Jhurani, B.B. Aggarwal, Multi-targeted therapy by curcumin: how spicy is it? Mol. Nutr. Food Res. 52 (9) (2008) 1010–1030.
[4] G.G. Mackenzie, N. Queisser, M.L. Wolfson, C.G. Fraga, A.M. Adamo, P.I. Oteiza, Curcumin induces cell-arrest and apoptosis in association with the inhibition of constitutively active NF-kappaB and STAT3 pathways in Hodgkin's lymphoma cells, Int. J. Canc. 123 (1) (2008) 56–65.
[5] D. Peer, J.M. Karp, S. Hong, O.C. Farokhzad, R. Margalit, R. Langer, Nanocarriers as an emerging platform for cancer therapy, Nat. Nanotechnol. 2 (12) (2007) 751–760.
[6] W. Mehnert, K. Mader, Solid lipid nanoparticles: production, characterization and applications, Adv. Drug Deliv. Rev. 47 (2–3) (2001) 165–196.
[7] A. Fundaro, R. Cavalli, A. Bargoni, D. Vighetto, G.P. Zara, M.R. Gasco, Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats, Pharmacol. Res. : the official journal of the Italian Pharmacological Society 42 (4) (2000) 337–343.
[8] G.P. Zara, R. Cavalli, A. Bargoni, A. Fundaro, D. Vighetto, M.R. Gasco, Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues, J. Drug Target. 10 (4) (2002) 327–335.
[9] S.K. Jain, A. Chaurasiya, Y. Gupta, A. Jain, P. Dagur, B. Joshi, V.M. Katoch, Development and characterization of 5-FU bearing ferritin appended solid lipid nanoparticles for tumour targeting, J. Microencapsul. 25 (5) (2008) 289–297.
[10] L.H. Reddy, J.S. Adhikari, B.S. Dwarakanath, R.K. Sharma, R.R. Murthy, Tumoricidal effects of etoposide incorporated into solid lipid nanoparticles after

intraperitoneal administration in Dalton's lymphoma bearing mice, AAPS J. 8 (2) (2006) E254–E262.
[11] R. Zhu, K.W. Cheng, G. Mackenzie, L. Huang, Y. Sun, G. Xie, K. Vrankova, P.P. Constantinides, B. Rigas, Phospho-sulindac (OXT-328) inhibits the growth of human lung cancer xenografts in mice: enhanced efficacy and mitochondria targeting by its formulation in solid lipid nanoparticles, Pharmaceut. Res. 29 (11) (2012) 3090–3101.
[12] P. Wang, L. Zhang, H. Peng, Y. Li, J. Xiong, Z. Xu, The formulation and delivery of curcumin with solid lipid nanoparticles for the treatment of on non-small cell lung cancer both in vitro and in vivo, Mater Sci Eng C Mater Biol Appl 33 (8) (2013) 4802–4808.
[13] R. Arora, A. Kuhad, I.P. Kaur, K. Chopra, Curcumin loaded solid lipid nanoparticles ameliorate adjuvant-induced arthritis in rats, Eur. J. Pain 19 (7) (2015) 940–952.
[14] H. Rachmawati, A.T. Pradana, D. Safitri, I.K. Adnyana, Multiple functions of d-alpha-tocopherol polyethylene glycol 1000 succinate (TPGS) as curcumin nanoparticle stabilizer: in vivo kinetic profile and anti-ulcerative colitis analysis in animal model, Pharmaceutics 9 (3) (2017) E24.
[15] H. Rachmawati, D. Safitri, A.T. Pradana, I.K. Adnyana, TPGS-stabilized curcumin nanoparticles exhibit superior effect on carrageenan-induced inflammation in wistar rat, Pharmaceutics 8 (3) (2016) E24.
[16] M.V. Varma, R. Panchagnula, Enhanced oral paclitaxel absorption with vitamin E-TPGS: effect on solubility and permeability in vitro, in situ and in vivo, Eur. J. Pharmaceut. Sci. 25 (4–5) (2005) 445–453.
[17] B.M. Andjelic, B.S. Mihaljevic, L.R. Jakovic, ABVD as the treatment option in advanced Hodgkin's lymphoma patients older than 45 years, Pathol. Oncol. Res. 18 (3) (2012) 675–680.
[18] S. Viviani, P.L. Zinzani, A. Rambaldi, E. Brusamolino, A. Levis, V. Bonfante, U. Vitolo, A. Pulsoni, A.M. Liberati, G. Specchia, P. Valagussa, A. Rossi, F. Zaja, E.M. Pogliani, P. Pregno, M. Gotti, A. Gallamini, D. Rota Scalabrini, G. Bonadonna, A.M. Gianni, F. Michelangelo, L. Gruppo, Italiano di Terapie Innovative nei, L. Intergruppo Italiano, ABVD versus BEACOPP for Hodgkin's lymphoma when high-dose salvage is planned, N. Engl. J. Med. 365 (3) (2011) 203–212.
[19] F.L. Meyskens Jr., C.E. McLaren, D. Pelot, S. Fujikawa-Brooks, P.M. Carpenter, E. Hawk, G. Kelloff, M.J. Lawson, J. Kidao, J. McCracken, C.G. Albers, D.J. Ahnen, D.K. Turgeon, S. Goldschmid, P. Lance, C.H. Hagedorn, D.L. Gillen, E.W. Gerner, Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial, Canc. Prev. Res. 1 (1) (2008) 32–38.
[20] S.C. Gupta, S. Patchva, B.B. Aggarwal, Therapeutic roles of curcumin: lessons learned from clinical trials, AAPS J. 15 (1) (2013) 195–218.
[21] S.C. Gupta, S. Patchva, W. Koh, B.B. Aggarwal, Discovery of curcumin, a component of golden spice, and its miraculous biological activities, Clin. Exp. Pharmacol. Physiol. 39 (3) (2012) 283–299.
[22] S. Rahman, S. Cao, K.J. Steadman, M. Wei, H.S. Parekh, Native and beta-cyclodextrin-enclosed curcumin: entrapment within liposomes and their in vitro cytotoxicity in lung and colon cancer, Drug delivery 19 (7) (2012) 346–353.
[23] T. Tanaka, M. Narazaki, T. Kishimoto, IL-6 in inflammation, immunity, and disease, Cold Spring Harbor perspectives in biology 6 (10) (2014) a016295.
[24] G.M. Reynolds, L.J. Billingham, L.J. Gray, J.R. Flavell, S. Najafipour, J. Crocker, P. Nelson, L.S. Young, P.G. Murray, Interleukin 6 expression by Hodgkin/Reed-Sternberg cells is associated with the presence of 'B' symptoms and failure to achieve complete remission in patients with advanced Hodgkin's disease, Br. J. Haematol. 118 (1) (2002) 195–201.
[25] J. Gopas, E. Stern, U. Zurgil, J. Ozer, A. Ben-Ari, G. Shubinsky, A. Braiman, R. Sinay, J. Ezratty, V. Dronov, S. Balachandran, D. Benharroch, E. Livneh, Reed-Sternberg cells in Hodgkin's lymphoma present features of cellular senescence, Cell Death Dis. 7 (11) (2016) e2457.
[26] A. Gallamini, C. Tarella, S. Viviani, A. Rossi, C. Patti, A. Mule, M. Picardi, A. Romano, M. Cantonetti, G. La Nasa, L. Trentin, S. Bolis, D. Rapezzi, R. Battistini, D. Gottardi, P. Gavarotti, P. Corradini, M. Cimminiello, C. Schiavotto, G. Parvis, R. Zanotti, G. Gini, A.J.M. Ferreri, P. Viero, M. Miglino, A. Billio, A. Avigdor, A. Biggi, F. Fallanca, U. Ficola, M. Gregianin, A. Chiaravalloti, G. Prosperini, F. Bergesio, S. Chauvie, C. Pavoni, A.M. Gianni, A. Rambaldi, Early chemotherapy intensification with escalated BEACOPP in patients with advanced-stage Hodgkin lymphoma with a positive interim positron emission tomography/computed tomography scan after two ABVD cycles: long-term results of the GITIL/FIL HD 0607 trial, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 36 (5) (2018) 454–462.
[27] S.C. Kamran, H.A. Jacene, Y.H. Chen, P.M. Mauch, A.K. Ng, Clinical outcome of patients with early stage favorable Hodgkin lymphoma treated with ABVD x two cycles followed by FDG-PET/CT restaging and 20 Gy of involved-site radiotherapy, Leuk. Lymphoma (2017) 1–7.
[28] N. Skoetz, A. Will, I. Monsef, C. Brillant, A. Engert, B. von Tresckow, Comparison of first-line chemotherapy including escalated BEACOPP versus chemotherapy including ABVD for people with early unfavourable or advanced stage Hodgkin lymphoma, Cochrane Database Syst. Rev. 5 (2017) CD007941.
[29] K.C. Marr, J.M. Connors, K.J. Savage, K.J. Goddard, R.J. Deyell, ABVD chemotherapy with reduced radiation therapy rates in children, adolescents and young adults with all stages of Hodgkin lymphoma, Ann. Oncol. : official journal of the European Society for Medical Oncology / ESMO 28 (4) (2017) 849–854.
[30] Y. Jiang, Y. Chen, R. Huang, G. Chen, Comparison of the efficiency of ABVD versus BEACOPP for Hodgkin lymphoma treatment: a meta-analysis, Int. J. Hematol. 104 (4) (2016) 413–419.
[31] T.C. Chou, Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies, Pharmacol. Rev. 58 (3) (2006) 621–681.