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ANTICANCER-LOADED SOLID LIPID NANOPARTICLES: HIGH POTENTIAL ADVANCEMENT IN CHEMOTHERAPY

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The enhancement in cancer chemotherapy through incorporation into solid lipid nanoparticles (SLN) drug delivery system carries a lot of potential. Improving tumor diffusivity, improvement of body distribution, enhancing cellular uptake and inhibiting multidrug resistance mechanism are the main attributes. The availability of variety of methods of preparation with scale up possibility and the higher biocompatibility of elements used may accelerate their arrival to the pharmaceutical market. Nanostructured lipid carriers (NLC) and lipid hybrid nanoparticles provide options to overcome some of SLN limitations. This review provides a thorough update on the development in SLN optimization toward chemotherapy improvement.

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1. Introduction

Cancer is one of the leading causes of death worldwide, second only to heart diseases. One in 4 deaths in the US is due to cancer. According to the National Cancer Institute, more than 12 million men and women in the United States, in 2009, were alive who had a history of cancer of all sites and the mortality rate from 2005 to 2009 were 370 per each 100,000 person [1, 2]. The early detection of cancer is considered the critical step in improving cancer treatment. When cancer is diagnosed at an early stage, treatment is often simpler and more likely to be effective. So finding cancer early can make a real difference. Chemotherapy is considered an important treatment modality in cancer and will probably remain so for considerable time. However, systemic administration of most drugs for cancer therapy produces severe side-effects due to their cytotoxic effects on normal cells. Therefore, significant efforts have been made to develop novel targeted delivery systems that can provide higher specificity to cancer cells with no/minimal effect on normal cells.

Nanotechnology has become a rapidly growing field with potential applications in health and drug therapy [3-5]. Nanoparticles have extraordinary physical and chemical properties resulting from the nanosize effect [6]. The small size and the high surface area to volume ratio are among the most featured nanoproperties. These can be exploited for the attachment with many functional groups that can seek out for specific targeting to certain cancer cells.

Nanomedicine can be defined as the translation of all positive signals of nanotechnology to be applied in the improvement of diseases therapy. Many new commercial applications of nanomedicine in the pharmaceutical industry have been stated by NNI. They include drug delivery, in vivo imaging, and Neuro-electronic interfaces and other nanoelectronics-based sensors.

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2. Overview of current cancer treatments

Cancer is usually treated with a combination of surgery, chemotherapy and/or radiotherapy. Chemotherapy is the general term for any treatment involving the use of chemical agents to stop cancer cells from growing. Chemotherapy can eliminate cancer cells at sites great distances from the original cancer. As a result, chemotherapy is considered a systemic treatment. Cytotoxic drugs are a diverse class of compounds that treat cancer primarily by being toxic to cells that are rapidly growing and dividing. Cytotoxic drugs are conventionally administered by intravenous bolus or infusion, typically in the form of free drug solutions.

Chemotherapy has played a major role in cancer treatment for more than half a century. More than half of all people diagnosed with cancer receive chemotherapy. For millions of people, chemotherapy helps treat their cancer effectively, enabling them to enjoy full, productive lives. Chemotherapy works by killing rapidly dividing cells including cancer cells and rapid proliferating normal cells such as bone marrow, gastrointestinal tract, reproductive system and hair follicles [7]. Healthy cells usually recover shortly after chemotherapy is complete. Unlike radiation, chemotherapy treats cancer on a cellular basis throughout the entire body. As a result, any cells that may have broken away from the original cancer are treated. In addition, chemotherapy plays a vital role in cancer palliative therapy to enhance the quality of life for patients. Chemotherapy will probably remain an important treatment modality for considerable time [8].

2.1 Shortcoming to conventional cancer chemotherapy:

Despite the long history of the clinical use of chemotherapy, the outcome remains unsatisfactory. Many solid tumors have presented low response rate to chemotherapy. These malegnancies include pancreatic cancer, ovarian cancer, esophageal cancer, and breast cancer [7].

The conventional administration of chemotherapeutic agents usually results in wide biodistribution and allows binding to different tissues and plasma proteins. Consequently, a small fraction only reaches cancer tissues [9]. The ability of cytotoxic drugs to bind specifically to tumor tissues is very poor and this is considered a major challenge to effective anticancer treatment. The general toxicity profile of cytotoxic drugs is mainly induced by attacking of normal rapid proliferating cells such as in bone marrow, gastrointestinal mucosa, gonadal tissue, and hair follicles [10-12]. This leads to side effects such as severe anemia, vomiting, nausea, hair loss, and fatigue which are commonly caused by most chemotherapeutic drugs. In addition, some cytotoxic agents induce their own specific toxicities on certain tissues or organs. The cardiotoxicity induced by doxrobucin, the nephro toxicity induced by cisplatin, and the lung toxicity caused by bleomycin are some examples [13-16]. Since both specific and non-specific adverse effects of such drugs are dose dependents [17]. The use of high dose intensity to ensure therapeutic success is usually accompanied by a high risk of normal tissue toxicity.

Another important barrier to efficient anticancer chemotherapeutic treatment is the specific cancer cells defense mechanisms at the cellular level. The active efflux of a broad range of cytotoxic drug molecules out of the cytoplasm by membrane bound transporters is most important factor and is known as the multidrug resistance MDR [18, 19]. Multidrug transporter expression is considered the key factor responsible for the arising of tumor cell resistance to anticancer therapy and reducing disposition of chemotherapy drugs inside tumor cells. Subsequently, this leads to the need of higher doses in order to induce substantial anticancer activity resulting in higher associated chemotherapy toxicity [20]. Resistance may be restricted to certain anticancer agents and then is named individual drug resistance [21]. Molecularly targeted therapy has emerged as one approach to overcome the lack of specificity of conventional chemotherapeutic agents [22]. However, the development of resistance in cancer cells can evade the cytotoxicity not only of conventional chemotherapeutics but also of this newer molecularly targeted therapeutics [23].

However, in most cases, resistance to cytotoxic compounds is associated with cross-resistance to different drugs with or without structural similarity to the primary agent.

Conclusively, the main obstacles to conventional chemotherapy are the poor specificity, high toxicity and susceptibility to induce drug resistance. Therefore, significant efforts have been

made to develop novel targeted delivery systems that can provide higher specificity to cancer cells with no/minimal effect on normal cells.

2.2 Potential benefits of nanomedicine in cancer therapy:

Cancer Nanotechnology is a new interdisciplinary research area aiming to utilize the integration between chemistry, biology, engineering and medicine toward marked advances in cancer diagnosis and treatment [24-27]. Since the application of nanotechnology to the imaging of gliomas was proposed, there has been a rapid expansion of the application of nanodevices to the diagnosis and treatment of brain tumors [28].

One of the potential advantages of incorporating anticancer agents into nanoparticles is to enhance their cellular uptakes by bypassing the different multi drug resistant mechanisms. Also they are excellent tumor-targeting vehicles because of a unique inherent property of solid tumors. Due to the rapid growth of solid tumors, many tumors grow with fenestrated vasculature and poor lymphatic drainage, resulting in an enhanced permeability and retention (EPR) effect [29], which allows nanoparticles to accumulate specifically at the tumor site. Nanoparticles can protect drugs from rapid metabolism and clearance. In addition, they can be designed to avoid nonspecific recognition and distribution including; uptake by the reticuloendothelial (mononuclear phagocytic) system [30, 31] leading to prolong circulating nanoparticles in the body, allowing them to eventually reach the tumor vasculature where, guided by the EPR effect, they specifically extravasate through the fenestrated capillaries to accumulate drugs at the tumor mass.

Beyond the passive tumor-targeting properties by the EPR effect, intratumoral localization of nanoparticles can be further improved by active targeting through conjugation of the particle with tumor-specific recognition of small molecules, such as folic acid [32], thiamine [33] and even antibodies or lectins [34]. In addition, at the tumor site, nanoparticles offer one further advantage: they can be endocytosed/phagocytized, enhancing cell internalization of the drug, and leading to delivery of the drug closer to the intracellular site of action [30].

The main drawbacks that have limited the wide spread application of nanoparticles to clinical medicine are the scarcity of safe polymers with regulatory approval and their high cost [35].

3. Solid lipid nanoparticles:

Solid lipid nanoparticles (SLN) are type of nanoparticulate drug delivery system in which the drug carrier is a lipid that solidifies at room temperature. They were first described by Müller et al. [36] and since that date, they brought a great deal of attention as novel drug delivery carrier [37].

They differ than nanoemulsion only in that liquid lipids (oils), used in the preparation of nanoemulsions, are substituted with solid lipids [38, 39]. The majority of lipids commonly used are triglyceride esters of hydrogenated fatty acids. Hydrogenated cottonseed oil (Lubritab™ or Sterotex™), hydrogenated palm oil (Dynasan™ P60 or Softisan™ 154), hydrogenated castor oil (Cutina™ HR), and hydrogenated soybean oil (Sterotex™ HM, or Lipo™) are typical examples [40]. SLN can be utilized in number of applications including; enhancing drugs solubility [41], controlling drug release, drug targeting [35, 42, 43], reduction in therapeutic dose, enhanced bioavalability [44], and increased stability of the drug [35, 43]. SLN have been suggested for administration by number of routes such as peroral, parenteral, topical [35, 42, 45], and pulmonary [42, 45].

SLN were introduced as a novel drug carrier system for oral delivery in the middle of 1990s [35]. The adhesive properties of nanoparticles are reported to increase bioavailability and reduce or minimize erratic absorption [46]. Absorption of nanoparticles occurs through mucosa of the intestine by several mechanisms namely through the Peyer's patches, by intracellular uptake or by the paracellular pathway. Pinto and Muller [47] incorporated SLN into spherical pellets and investigated SLN release for oral administration. SLN granulates or powders can be put into capsules, compressed into tablets or incorporated into pellets. The stability of SLN upon contact

with GI fluids is a critical issue since particle size in the nano range maximizes the surface area for enzymatic degradation [48].

3.1 Advantages of SLN

SLN offers number of beneficial attributes over the other colloidal drug delivery systems. These include:

- Under optimized conditions they can incorporate lipophilic or hydrophilic drugs [49, 50].
- Their colloidal dimensions and the controlled release behavior enable drug protection and administration by parenteral and non-parenteral routes [42, 45].
- No hazard in vivo fate as they are composed mainly of physiological lipids [51].
- They comprise higher permeability through the BBB i.e. suitable for targeting drugs to the brain [52].
- Low production cost compared with liposomes.
- They exhibit good stability during long-term storage and are amenable to both lyophilization and steam sterilization [53].
- SLN confers improved protein stability and avoids proteolytic degradation [54].
- They can be prepared with techniques employed in industry such as High pressure homogenization (HPH) and supercritical fluid (SCF) technology [49, 50].

3.2 Drawbacks of SLN:

The main drawbacks of SLN include:

- Poor drug loading capacity, the drug loading capacity of conventional SLN is limited by the solubility of drug in the lipid melt [45, 50].
- Drug expulsion during storage as a result of the ability of some lipids to form perfect crystalline lattice with few imperfections [45].
- The relatively high water content of the dispersions (70-99.9%) has been observed [55].

3.3Nanostructured lipid carriers (NLC):

In an attempt to overcome the above mentioned SLN drawbacks, NLC were introduced [56-58]. Three approaches were employed aiming to increase the drug loading and prevent drug expulsion. The incorporation of small amounts of oils with the solid lipid is called imperfect type NLC. The added oil will increase the imperfection in the crystal lattice of the lipid and thus avoiding the expulsion of the loaded drug to the surface. Another approach is the use of combinations of spatially different lipids. This leads to larger distances between the fatty acid chains of the glycerides and general imperfections in the crystal providing more room for accommodation of guest molecules. A third approach is proposed based on avoiding crystallization of lipids upon cooling by mixing special lipids like hydroxyl octacosanyl, hydroxyl stearate and isopropyl myristate.

The NLCs have mainly been extensively investigated in the delivery of drugs such as clotrimazole [59, 60], ketoconazole [61], and other antifungal imidazoles [62].

4. Methods of sln preparation

SLNs are made up of solid lipid, emulsifier and water/solvent. The two phases (lipid and aqueous) should be thoroughly mixed to form one homogenous phase (emulsion) with droplet size in the nano range. Then, the particles are allowed to solidify by cooling or solvent evaporation based on the employed method of preparation. According to the drug solubility, the type of emulsion is determined and accordingly, the emulsifier(s) were chosen. Many types of lipids were used including triglycerides, partial glycerides, fatty acids, steroids and waxes. Various emulsifiers and their combinations have been used to stabilize the lipid dispersion. Combination of emulsifiers might prevent particle agglomeration more efficiently [63].

4.1 Micro emulsion based SLN preparation:

Normally, microemulsion is prepared by mixing a liquid lipid, surfactant, and mostly co-surfactant with water in a certain ratio predetermined using phase diagram. The same method will be employed here using a high temperature enough to keep the lipid in the melted state. The mixture is dispersed in cold water under mild mixing to allow the precipitation of SLN.

Number of publications described the procedures of preparing SLN through microemulsion approach [64, 65]. Simply, the lipid is melted; a mixture of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipid and added under mild stirring to the lipid melt. The amount of each component was adjusted to form a transparent, thermodynamically stable system based on the preliminary phase diagram data. The formed microemulsion is then dispersed in a cold aqueous medium (2-3ºC) under mild mechanical mixing. This may lead to undesirable dilution of SLN dispersion.

4.2 Ultrasonication or high speed homogenization:

This method depends on the formation of emulsion with droplet size in the nano range using either High speed homogenization or ultrasonication as dispersing techniques [66, 67]. Unlike the microemulsion, no preliminary phase diagram is needed. Ahlin et al. [68] optimized the process conditions such as emulsification time, stirring rate, and cooling time and correlated them with particle size and zeta-potential. The parameters that produce the best SLN quality were stirring for 5- 10 minutes at 20,000 to 25,000 rpm using 5 to 10 min cooling at 5000 rpm in cold water at room temperature [35]. The average particle sizes in the range of 100–200 nm were obtained using these conditions [35].

The main limitation of high speed homoginization method is the wide range of particle size distribution and the possibility of particle growth upon storage. Potential metal contamination due to ultrasonication is also a big problem in this method.

4.3 High Pressure homogenization:

High pressure homogenization (HPH) uses a high pressure (100-2000 bar) [35, 42] to push lipids though a narrow gap, in the range of a few microns, which cause the decrease in the size of the particles [42, 69]. One of the advantages of this method is that it does not show problems when scaling up [35]. Simply, the drug is dissolved or dispersed in the melted lipids (5-10°C above its melting point) and then emulsified in hot surfactant solution using high shear mixing for short period. Then, the hot nanoemulsion will be subjected to high pressure for number of cycles (3-5). In cold homogenization the final emulsion will be suddenly cooled by liquid nitrogen, while in hot homogenization it will be allowed to cool gradually at room temperature [42, 69].

The hot homogenization technique would decrease the viscosity of the inner phase which will result in lower particle size [42]. However unlike the cold method, the possible increase in the rate of degradation of the drug and the carrier and high potential for burst release are main disadvantages of the hot method [42, 69].

4.4 Solvent emulsification/evaporation:

Simply, a lipid is dissolved in an organic solvent immiscible with water and then the drug aqueous solution containing surfactant and/or co-surfactant is emulsified in the organic phase. The SLN will be obtained upon the evaporation of the organic solvent under continuous stirring [70].

Siekmann and Westesen [71] described a method used to incorporate cholesterol acetate (a model drug) in SLN prepared by precipitation in O/W emulsions using cyclohexan and lecithin/sodium glycocholate blend as emulsifier. Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The mean diameter of the obtained particles was 25 – 29 nm with very good reproducibility.

4.5 Double emulsion method

This technique is extensively used in the literature for the production of SLN and polymeric nanoparticle especially for hydrophilic drugs [72, 73]. This method is considered a modification to solvent emulsification-evaporation method with the application of a stabilizing hydrophilic coat over the lipid layer [74]. Yassin et al. [75] prepared 5-FU loaded SLN by double emulsion-solvent evaporation technique (w/o/w) using triglyceride esters, Dynasan™ 114 or Dynasan™ 118 along with soyalecithin as the lipid parts. Three optimized SLNs formulae have been successfully prepared with particle size in the range of 300 -400 nm.

4.6 SLN preparation by supercritical fluid Technology:

This is a relatively new technique for SLN production and has the advantage of the ability for scale-up production in industry. Two types of supercritical fluid instruments can be applied in the production of SLN; the rapid expansion of supercritical carbon dioxide solutions (RESS) and supercritical antisolvent system (SAS). In RESS, the drug and lipid are dissolved in carbon dioxide (99.99%), while in SAS, cabon dioxide is used as antisovent to the drug and lipid and solvent for the organic solvent in which they are dissolved [76]. The main advantage of this method is the ability to control the particle size and shape to high quality and the solvent-less processing [77, 78].

4.7 Spray drying method:

Spray-drying technique is extensively used in the pharmaceutical industry to produce raw drug or excipients or microparticles, as an alternative to emulsification methods [79, 80]. Spray-drying can be utilized in the production of SLN as a substituent to freeze-drying by direct conversion of the SLN aqueous or alcoholic dispersion to reconstitutable particles. The process can be optimized to obtain uniform particle size and easily redispersed SLN by using alcoholic dispersions to reduce the temperature, reducing the lipid concentration while increasing the lyoprotectant sugar concentration, and by redispersion in surfactant solution [48]. Recently, a nano-spraydryer has been introduced for the production of nanoparticles. This technique may open new horizons in the development of SLN.

5. SLN as delivery moiety for anticancers

The cytotoxicity of many chemotherapeutic agents was compared when loaded in SLN with their conventional therapeutic forms. In one study, the cytotoxicity of SLN formulations carrying cholesteryl butyrate, doxorubicin (Dox) or paclitaxel (PTX) were evaluated on the human colorectal cancer cell line HT-28 [81]. The results showed that SLN of cholesteryl butyrate and Dox exhibited significantly higher cytotoxicities than the equivalent amount of free drug. The 50% inhibitory concentration (i.e. IC_{50}) values for HT-28 cell growth of SLN drug formulations were both lower than the corresponding conventional drug solutions (butyrate: 0.3 mM versus 0.6 mM; Dox: 81.87 nM versus 126.57 nM respectively). However, PTX loaded SLN showed almost similar cytotoxic compared to the equivalent amount of drug in free solution containing cremophor EL. This was ascribed to the poor water solubility of PTX, which results in low drug release from the SLN [82, 83]. The substitution of the toxic cremophor EL with SLN was considered beneficial. In another study, the in vivo efficacy of PTX loaded SLN was compared with free drug formulation using murine breast cancer mice model [84]. It was found that the group of animal treated with PTX loaded SLN had significantly smaller tumor size and lower percent inhibition ($P<0.05$).

Recently, Travera et al. [85] were able to construct cationic SLN made of stearic acid and gylceryl behenate 1:2 using Poloxamer and cetylpyridinium chloride as surfactant and co-surfactant, respectively. The optimized formula allowed 97% loading of Dox and showed significant increase in cytotoxicity in B16F10 murine melanoma culture cell line.

The ability of SLN to protect a new topoisomerase inhibitor prodrug SN-38 was using a mice model xenografted with HT-29 tumor [86]. Animals with tumors treated with the SLN formulation of SN-38 took longer or comparable times to reach the cut off tumor weight (1 g) at a lower drug dose when compared to free drug solution. It seems that SLN kept the drug from undergoing lactone ring hydrolysis, which highly correlated with the anticancer activity of this class of drugs, until it was released from SLN [86].

Methotrexate- loaded SLNs, prepared by coacervation, showed an increased cytotoxicity towards MCF-7 and Mat B-III cell lines compared with free drug [87]. The in vivo animal study showed that after intravenous administration, higher blood levels were achieved and major drug accumulation within breast cancer tumor tissue was shown compared with drug solution alone. In another study, methotrexate loaded SLN was prepared and assessed after IV administration to EAC (Ehrlich Ascite Carcinoma) bearing mice [88]. Compared with methotrexate solution, higher MRT (mean residence time) and elimination half-life was reported. In addition, significant increase in the life span of the group of animals treated with methotrexate loaded SLN was documented.

The ability of SLN to enhance the CNS targeting of lipophillic anticancers was assessed using camptothecin in mice [52]. The pharmacokinetics of camptothecin after IV administration to two groups of animals; one group received camptothecin solution while the other group received camptothecin loaded SLN. The concentrations of camptothecin in various organs were determined using reversed-phase high-performance liquid chromatography. The results showed that the AUC/dose and MRT of loaded SLN were much higher than those of camptothecin solution, especially in brain, heart and reticuloendothelial cells containing organs. This may allow a reduction in dosage and a decrease in systemic toxicity of some anticancers.

5.1 Effect on multidrug resistance mechanism

Doxorubicin- loaded polymer lipid hybrid nanoparticles (PLN) were evaluated on a murine breast cancer cell line EMT6/AR1 and a human breast cancer cell line MDA435/LCC6/MDR1 [89, 90]. Both cell lines carry multidrug resistance (MDR) phenotype as a result of P-glycoprotein (P-gp) over expression. A clonogenic assay was carried out to evaluate the anticancer activities of the Dox-loaded PLN by measuring the fraction of drug-treated cancer cells that is able to proliferate to form viable colonies. The assays showed that Dox-loaded PLN resulted in over 8-fold increase in MDR cancer cell (EMT6/AR1 cell line) kill when compared to free solution of Dox at equivalent doses. Further investigations showed that the MDR cancer cells accumulated and retained PLN-loaded Dox at substantially higher levels than Dox solution [90]. This indicates the ability of PLN to alter the MDR.

The ability of SLN to inhibit the Pgp as a main multidrug resistance in cancer therapy was investigated [91]. Dox loaded SLN were previously prepared by solvent emulsification-diffusion method using glyceryl caprate and curdlan [92]. Compared with free Dox, the prepared SLNs did not show hemolytic activity in human erythrocytes and efficiently enhanced apoptotic cell death through enhancement in the cellular uptake through P-gp-overexpressing MCF-7/ADR cells, a representative Dox-resistant breast cancer cell line [91].

5.2 Antiadhesive mechanism

Minelli et al. [93] have prepared cholesteryl butyrate SLN aiming to enhance the adhesion on cancer cell since cancer cell adhesion to endothelium is crucial for metastasis dissemination. They incubated the cholesteryl butyrate SLN with cancer or endothelial cells and adhesion was quantified by a computerized micro-imaging system. Migration was detected by the scratch "wound healing" assay and the Boyden chamber invasion assay. Expression analysis of ERK (extracellular regulatory kinase) and p38 MAPK (the mitogen-activated protein kinases) was performed by Western blot. They found that SLN may act as an anti-metastastic drug, and they add a novel mechanism to the anti-tumour activity of this multifaceted drug.

6. Conclusion

SLN based drug delivery carries many attributes toward improving chemotherapy through enhancing the cellular uptake of many cancer cells and alter the MDR and allowing thus allowing reduction in the dose related toxicity of anticancers. The CNS targeting ability of SLN would have impact on improving chemotherapy of brain tumors. The flexibility in the methods of preparations and the simplicity of large scale production may encourage the wide spread use of SLN generally and particularly for cancer therapy. It is expected that the near future will witness tremendous expansion of the role of SLN based drug delivery in cancer therapeutics.

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose. This review received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

[1] National Cancer Institute, cancer Stat Fact Sheet, available on Web Site: http://seer.cancer.gov/statfacts/html/all.html.
[2] N. Howlader, A. Noone, M. Krapcho et al, Eds. *SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations)*, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2009_pops09/, based on November 2011 SEER data submission, posted to the SEER web site, 2012.
[3] S. Shoo, S. Parveen, J. Panda, Nanotechnology **3**, 20 (2007).
[4] J. Park, S, Lee, J. Kim et al, Polymeric nanomedicine for cancer therapy. Prog. Polym Sci **33**,113 (2008).
[5] B. Sumer, J. Gao, Therapeutic nanomedicine for cancer. Nanomedicine **3**, 137 (2008).
[6] A. Kabanov, H. Gendelman, Nanomedicine in the diagnosis and therapy of neurodegenerative disorders. Prog. Polym Sci **32**, 1054 (2007).
[7] R. B. Ewesuedo, M.J. Ratain, Principles of cancer chemotherapy. In: E Vokes, H Golomb. Eds., Oncologic Therapeutics, Springer, New York (2003).
[8] P. Nygren, What is cancer chemotherapy? Acta Oncologica, **40**,166 (2001).
[9] M. Ratain, R. Mick, Principles of pharmacokinetics and pharmacodynamics. In: Schilsky RL, Milano GA, Ratain MJ. Eds. Principles of Antineoplastic Drug Development and Pharmacology, Marcel Dekker, New York (1996).
[10] G. Powis, A unique opportunity to study human toxicology. In: G. Powis G, Hacker MP. Eds. The Toxicity of Anticancer Drugs, Pergamon Press, Toronto (1991).
[11] J. Tipton, Side effects of cancer chemotherapy. In: Skeel RT. Ed. Handbook of Cancer Chemotherapy, Lippincott Williams & Wilkins, Philedelphia (2003).
[12] S. Kummar, M. Gutierrez, J. Doroshow, A. Murgo. Drug development in oncology: classical cytotoxics and molecularly targeted agents. Br. J Clin Pharmacol **62**, 15 (2006).
[13] B. Kalyanaraman, J. Joseph, S. Kalivendi S *et al*, Doxorubicin-induced apoptosis: implications in cardiotoxicity, Mol. Cell Biochem **234**,119,(2002).
[14] V. Garipidou, S. Vakalopoulou , E. Zafiriadou E *et al*, Uncommon manifestation of bleomycin-induced pulmonary toxicity in a patient with Hodgkin's disease. Annals. of Oncology **16**, 514 (2005).
[15] P. Lu. Monitoring cardiac function in patients receiving doxorubicin, Semin. Nucl Med **35**,197(2005).
[16] X. Yao, K. Panichpisal, N. Kurtzman, K. Nugent. Cisplatin Nephrotoxicity: A Review. Am. J Med Sci **334**,115 (2007).
[17] W. Hryniuk, A. Figueredo, M. Goodyear. Applications of dose intensity to problems in chemotherapy of breast and colorectal cancer. Sem. Oncol **11**, 3 (1987).

[18] R. Baird, S. Kaye. Drug resistance reversal—are we getting closer? Eur. J Cancer **39**, 2450 (2003).
[19] F. Gieseler, P. Rudolph, G. Kloeppel, U. R. Foelsch, Resistance mechanisms of gastrointestinal cancers: why does conventional chemotherapy fail? Int. J Colorectal Dis **18**, 470 (2003).
[20] A. Lockhart, R. Tirona, R. Kim R, Pharmacogenetics of ATP-binding cassette transporters in cancer and chemotherapy. Mol. Cancer Ther **2**, 685 (2003).
[21] D. Longley, P. Johnston. Molecular mechanisms of drug resistance. J. Pathol **205**, 275 (2005).
[22] J. Ross, D. Schenkein, R. Pietrusko *et al*, Targeted therapies for cancer 2004. Am. J Clin Pathol **122**,598 (*2004).*
[23] F. Morgillo, H. Lee, Resistance to epidermal growth factor receptor-targeted therapy. Drug Resist Update **8**, 298 (2005).
[24] P. Srinivas, P. Barker, S. Srivastava, Nanotechnology in early detection of cancer. Lab. Investig **82**, 657 (2002).
[25] M. Ferrari, Cancer nanotechnology: opportunities and challenges. Nat. Rev Cancer **5**,161(2005).
[26] S. Nie, Y. Xing, G. J. Kim, J. W. Simons, Nanotechnology applications in cancer. Annu. Rev Biomed Eng **9**, 257 (2007).
[27] M. Wang, D. Shin, J. Simons, S. Nie, Nanotechnology for targeted cancer therapy. Exp. Rev Anticancer Ther **7**, 833 (2007).
[28] C. Zimmer, R. Weissleder, K. Poss, A. Bogdanova, S. C. Wright, W. S. Enochs, MR imaging of phagocytosis in experimental gliomas. Radiology **197**,533(1995).
[29] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, Tumor vascular permeability and the EPR effect on macromolecular therapeutics. *a review.* J. Control Rel **65**, 271(2000).
[30] I. Brigger, C. Dubernet, P. Couvreur, Nanoparticles in cancertherapy and diagnosis. Adv. Drug Del Rev **54**, 631 (2002).
[31] L. Brannon-Peppas, J. Blanchette, Nanoparticle andtargeted systems for cancer therapy. Adv. Drug Del Rev **56**, 1649 (2004).
[32] J. Reddy, V. Allagadda, C. Leamon, Targeting therapeutic andimaging agents to folate receptor positivetumors. Curr. Pharm Biotechnol **6**, 131 (2005).
[33] Cascante M, Centelles J, Veech R, Lee W, Boros L. Role of thiamin (vitamin B1) and transketolase in tumor cell proliferation. Nutr. Cancer **36**,150 (2000).
[34] J. Park, C. Benz, F. Martin, Future directions of liposome and immunoliposome-based cancer therapeutics. Semin. Oncol **31**,196 (2004).
[35] W. Mehnert, K. Mader, Solid lipid nanoparticles Production, characterization and applications. Adv. Drug Deliv Rev **47**,165 (2001).
[36] R. Muller, W. Mehnert, J. Lucks *et al*, Solid lipid nanoparticles (SLN)-an alternative colloidal carrier system for controlled drug delivery. Eur. J Pharm. Biopharm **41**, 62 (1995).
[37] M. Jumaa, B, Muller, Lipid emulsions as a novel system to reduce the hemolytic activity of lytic agents: mechanism of protective effect. Eur. J Pharm Sci **9**, 285 (2000).
[38] R. Muller, C. Keck, Challenges and solutions for the delivery of biotech drugs – a review of drug nanocrystal technology and lipid nanoparticles. J. Biotech **113**,151 (2004).
[39] K. Manjunath, V. Venkateswarlu. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. J. Control Release **107**, 215 (2005).
[40] K. MacGregor, J. Embl eton, J. Lacy, et al. Influence of lipolysis on drug absorption from the gastro-intestinal tract. Adv. Drug Deliver Rev **25**, 33 (1997).
[41] S. Lim, C. Kim, Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid. Int. J Pharm *243,135(*2002).
[42] H. Jun, Z. Shi-wen, New research on development of solid lipid nanoparticles. Journal of Medical Colleges of PLA **22**,385 (2007).
[43] I. Kaur, R, Bhandari, S. Bhandari, V. Kakkar. Potential of solid lipid nanoparticles in brain targeting. J. Control Rel **127**, 97 (2008).
[44] M. A. Alex, A. Chacko, S. Jose, E. Souto, Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. Eur. J Pharm Sci **42**, 11 (2011).

[45] R. Muller, K. Mader, S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur. J Pharm Biopharm **50**,161(2000).
[46] G. Ponchel, M. Montisci, A. Dembri, C. Durrer, D. Duchêne, Mucoadhesion of colloidal particulate systems in the gastro-intestinal tract. Eur. J Pharm Biopharm **44**, 25 (1997).
[47] J. Pinto, R. Müller, Pellets as carriers of solid lipid nanoparticles (SLN) for oral administration of drugs. Pharmazie **54**, 506 (1999).
[48] C. Freitas, R. Mullera, Spray drying of solid lipid nanoparticles (SLN^{TM}). Eur. J Pharm Biopharm **46**,145(1998).
[49] R. Müller, M. Radtke, S. Wissing, Solid lipid nanoparticles and nanostructured lipid carriers. In: Nalwa HS, Ed. Encyclopedia of Nanoscience and Nanotechnology. Valencia, CA: American Scientific Publishers (2004).
[50] A. Almeida, E. Souto, Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Adv. Drug Deliv Rev **59**, 478 (2007).
[51] A. Rupenagunta, I. Somasundaram, V. Ravichandiram, J. Kausalya, B. Senthilnathan, Solid lipid nanoparticles-A versatile carrier system. J. Pharm Res **2**, 2069 (2011).
[52] S. Yang, L. Lu, Y. Cai *et al*, Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J. Control Release 59, 299 (1999).
[53] E. Souto, R. Müller, Lipid nanoparticles (SLN and NLC) for drug delivery. In: Domb A, Tobata Y, Kumar R, Farber S. Eds. Nanoparticles for Pharmaceutical Applications. Valencia, CA: American Scientific Publishers (2007).
[54] E. Souto, S. Doktorovová, Solid lipid nanoparticle formulations pharmacokinetic and biopharmaceutical aspects in drug delivery. Meth. Enzymol. **262**, 105 (2009).
[55] C. Schwarz, W. Mehnert, J. Lucks, R. Muller, Solid lipid nanoparticles (SLN) for controlled drug delivery I. Production, characterization and sterilization. J. Control Release **30**, 83 (1994).
[56] M. Radtke, R. Muller, Comparison of structural properties of solid lipid nanoparticles (SLN) versus other lipid particles. Proc. Int Symp Control Rel Bioact Mater **27**,309 (2000).
[57] R. Muller, M Radtke, S. Wissing , Nanostructured lipid matrices for improved microencapsulation of drug. Int. J Pharm **242**,121(2002).
[58] M. Uner, Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): Their benefits as colloidal drug carrier systems. Pharmazie **61**,375 (2006).
[59] E. Souto, S. Wissing, C. Barbosa, R. Muller, Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J Pharm **278**,71 (2004).
[60] E. Souto, R. Muller, Investigation of the factors influencing the incorporation of clotrimazole in SLN and NLC prepared by hot high-pressure homogenization. J. Microencapsul **23**,377 (2006).
[61] E. Souto, R. Muller, SLN and NLC for topical delivery of ketoconazole. J. Microencapsul **22**, 501(2005).
[62] E. Souto, R. Muller, The use of SLN and NLC as topical particulate carriers for imidazole antifungal agents. Pharmazie **61**,431 (2006).
[63] R. Cavalli, O. Caputo, M. Gasco. Solid lipospheres of doxorubicin and idarubicin. Int J Pharm **89**, R9 (1993).
[64] L. Boltri, T. Canal, P. Esposito, F. Carli, Lipid nanoparticles: evaluation of some critical formulation parameters, Proc. Int. Symp. Control. Release Bioact. Mater **20**, 346 (1993).
[65] M. Gasco, Solid lipid nanospheres from warm micro-emulsions, Pharm. Technol Eur **9**, 52 (1997).
[66] Speiser P. Lipidnanopellets als Tragersystem fur Arzneimittel zur peroralem Anwendung. European Patent No. EP 0167825(1990).
[67] T. Eldem, P. Speiser, A. Hincal, Optimization of spray-dried and congealed lipid microparticles and characterization of their surface morphology by scanning electron microscopy. Pharm. Res **8**, 47 (1991).
[68] P. Ahlin, J. Kristl, J. Sˇmid-Kobar, Optimization of procedure parameters and physical stability of solid lipid nanoparticles in dispersions, Acta Pharm **48**, 257 (1998).

[69] R. Parhi, P. Suresh, Production of Solid Lipid Nanoparticles-Drug Loading and Release Mechanism. J. Chem Pharm Res **2**, 211 (2010).
[70] B. Sjostrom, B. Bergenstahl, Preparation of submicron drug particles in lecithin-stabilized o/w emulsions: I: Model studies of the precipitation of cholesteryl acetate. Int. J Pharm **88**, 53 (1992).
[71] B. Siekmann, K. Westesen, Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions. Eur. J Pharm Biopharm **43**, 104 (1996).
[72] P. O'Donnell, J. McGinity. Preparation of microspheres by the solvent evaporation technique. Adv. Drug Deliv Rev **28**, 25 (1997).
[73] V. R Sinha, A. Trehan, Biodegradable microspheres for protein delivery. J. Control Release **90**, 261 (2003).
[74] R. Cortesi, E. Esposito, G. Luca, C. Nastruzzi, Production of lipospheres as carriers for bioactive compounds. Biomaterials **23**, 2283 (2002).
[75] A. Yassin, M.D Anwer, H. Mowafy *et al*, Optimization of 5-flurouracil solid-lipid nanoparticles: a preliminary study to treat colon cancer, Int. J Med Sci **7**, 398 (2010).
[76] P. Gosselin, R. Thibert, M. Preda, J. McMullen, Polymeric properties of micronized carbamazepine produced by RESS. Int. J Pharm **252**, 225 (2003).
[77] Chen et al. Preparation of solid lipid nanoparticles loaded with Xionggui powder-supercritical carbon dioxide fluid extraction and their evaluation in vitro release. Zhongguo Zhong Yao Za Zhi **31**, 376 (2006).
[78] C. Kaiser, H. Rompp, P. Schmidt, Pharmaceutical applications of supercritical carbon dioxide. Pharmazie **56**, 907 (2001).
[79] P. He, S. S. Davis, L. Illim, Chitosan microspheres prepared by spray drying. Int. J Pharm **187**, 53 (1999).
[80] A. Billon, B. Bataile, G. Cassanas, M. Jacob, Development of spray-dried acetaminophen microparticles using experimental designs. Int. J Pharm **203**,159 (2000).
[81] L. Serpe, M. Catalano, R. Cavalli *et al*, Cytotoxicity of anticancer drugs incorporated in solid lipid nanoparticles on HT-29 colorectal cancer cell line. Eur. J Pharm Biopharm **58**, 673 (2004).
[82] R. Cavalli, O. Caputo, M.R Gasco, Preparation and characterization of solid lipid nanospheres containing paclitaxel, Eur. J Pharm Sci **10**, 305 (2000).
[83] A. Singla, A. Garg, D. Aggarwal, Paclitaxel and its formulations, Int. J Pharm **235**, 179 (2002).
[84] Y. Zhuang, B. Xu, F. Huang, J. Wu , S. Chen, Solid lipid nanoparticles of anticancer drugs against MCF-7 cell line and a murine breast cancer model. Pharmazie **67**, 925 (2012).
[85] S. Taveira, L. Araújo, D. de Santana et al, Development of cationic solid lipid nanoparticles with factorial design-based studies for topical administration of doxorubicin. J. Biomed Nanotechnol **8**, 219 (2012).
[86] J. Williams, R. Lansdown, R. Sweitzer *et al*, Nanoparticle drug delivery system for intravenous delivery of topoisomerase inhibitors, J. Control Release **91**, 167 (2003).
[87] L. Battaglia, *L.* Serpe, *E. E.* Muntoni *et al*, Methotrexate-loaded SLNs prepared by coacervation technique: in vitro cytotoxicity and in vivo pharmacokinetics and biodistribution. Nanomedicine **6**,1561(2011).
[88] K. Ruckmani, M. Sivakumar, P. Ganeshkumar. Methotrexate loaded solid lipid nanoparticles (SLN) for effective treatment of carcinoma. J. Nanosci Nanotechnol **6**, 2991 (2006).
[89] H. Wong, R Bendayan, A Rauth *et al*, A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer-lipid hybrid nanoparticle (PLN) system. J. Pharmacol Exp Ther **317**,1372 (2006).
[90] H. Wong, R Bendayan, A Rauth *et al*, A new polymer-lipid hydrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug resistant human breast cancer cells. Pharm. Res **23**, 1574 (2006).
[91] K. Kang, M. Chun, O. Kim et al, Doxorubicin-loaded solid lipid nanoparticles to overcome multidrug resistance in cancer therapy. Nanomedicine. **6**, 210 (2010).
[92] R. Subedi, K. Kang, H. Choi, Preparation and characterization of solid lipid nanoparticles loaded with doxorubicin. Eur. J Pharm Sci **37**, 508 (2009).
[93] R. Minelli, L. Serpe , P. Pettazzoni *et al*, Cholesteryl butyrate solid lipid nanoparticles inhibit the adhesion and migration of colon cancer cells. Br. J Pharmacol 1**6**6, 587 (2012).