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Development of Nanocapsules Containing Cytotoxic Agents: A Review

(Kajian Literatur Pengembangan Sediaan Nanokapsul Mengandung Agen Sitotoksik)

Sani Ega Priani*, Tia Nur Setianty, Ratih Aryani, Sri Peni Fitrianingsih, Livia Syafnir

Department of Pharmacy, Universitas Islam Bandung (UNISBA), Bandung, Indonesia
Email : egapriani@gmail.com

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Corresponding Author:
Sani Ega Priani
Jurusan Farmasi
Fakultas MIPA
Universitas Islam Bandung
Bandung
40116
Indonesia
email: egapriani@gmail.com

ABSTRACT

Background: The incidence and mortality of cancer are rapidly growing worldwide. Modification on drug delivery systems based on nanotechnology was applied to improve the effectiveness and safety of treatment. Nanoencapsulation, a part of nanotechnology, was known can be involved in cytotoxic agents. **Objective**: This research was conducted to determine the type of polymers for nanoencapsulation of cytotoxic agents and analyze the effect of nanoencapsulation on the cytotoxic activity. **Methods**: The study was performed by systematic literature review using selected articles from reputable databases that meet the inclusion and exclusion criteria. **Results:** The results show that many cytotoxic agents have been developed in nanocapsules systems due to their low water solubility, chemical instability, and low bioavailability. The nanoencapsulation process was carried out using synthetic or natural polymers such as polylactic-co-glycolic acid (PLGA), PEGylated PLGA, polycaprolactone (PCL), chitosan-sodium tripolyphosphate, chitosan-sodium alginate, heparin-poly(l-lysine), and polymethyl methacrylate (PMMA). Those polymers are widely used for nanoencapsulation related to their biocompatible, biodegradable, non-toxic, and providing the desired coating properties. The nanoencapsulation on cytotoxic agents significantly increases the in vitro cytotoxicity, marked by the decrease of IC50 value in the range 1.4-15.4 folds compared to pure drugs. The increase in cytotoxicity could be caused by particle size reduction, modification of particle surface properties, and enhancement of drug stability. **Conclusion**: It can be concluded that nanoencapsulation can be applied for cytotoxic agents to increase their activity using the appropriate coating polymer.

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ABSTRAK

Latar Belakang: Angka kejadian dan kematian dari penyakit kanker terus berkembang pesat di seluruh Dunia. Modifikasi sistem penghantaran obat berbasis nanoteknologi diketahui bisa meningkatkan efektifitas dari terapi kanker. Nanoenkapsulasi sebagai salah satu bagian dari nanoteknologi diketahui dapat diterapkan untuk agen sitotoksik. **Tujuan**: Penelitian ini bertujuan untuk mengkaji jenis polimer yang sesuai digunakan untuk pengembangan nanokapsul mengandung agen sitotoksik dan menganalisis efek dari proses nanoenkapsulasi terhadap aktivitas sitotoksikn-nya. **Metode**: Penelitian dilakukan berbasis systematic literature review, dengan mengkaji artikel yang diperoleh dari database bereputasi, yang memenuhi kriteria inklusi dan eksklusi yang telah ditetapkan. **Hasil**: Hasil kajian menunjukkan bahwa sistem nanokapsul sudah diaplikasikan untuk banyak agen sitotoksik yang memiliki keterbatasan dalam hal kelarutan dalam air, stabilitas, dan biovaibilitas. Proses nanoenkapsulasi dapat dilakukan menggunakan polimer sintetik dan alami seperti *polylactic-co-glycolic acid*/ PLGA, *PEGylated PLGA*, *polycaprolactone* (PCL), kitosan-natrium tripolifosfat, kitosan-natrium alginat, *heparin- poly(l-lysine)*, dan *polymethyl methacrylate* (PMMA). Polimer tersebut banyak digunakan dalam proses nanoenkapsulasi karena sifatnya yang biokompatibel, biodegradable, non-toksik, dan dapat menghasilkan membran dengan karakteristik yang baik. Proses nanoenkapsulasi terhadap agen sitotoksik secara signifikan mampu meningkatkan aktivitas sitotoksik berdasarkan pengujian in vitro yang ditandai dengan penurunan nilai IC_{50} sebesar 1,4-15,4 kali bila dibandingkan dengan senyawa murninya. Peningkatkan aktivitas tersebut disebabkan karena sistem nanokapsul dapat menyebabkan penurunan ukuran partikel, perubahan sifat permukaan, dan peningkatan stabilitas senyawa. **Kesimpulan:** Proses nanoenkapsulasi dapat diaplikasikan untuk peningkatan aktivitas agen sitotoksik dengan penggunaan polimer penyalut yang sesuai.

Kata kunci: Nanokapsul, nanoenkapsulasi, sitotoksik, kanker, sistematik review

INTRODUCTION

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts (Sarkar et al., 2013). The incidence and mortality of cancer, rapidly growing worldwide. Based on WHO reports in 2019, cancer is the first or second leading cause of death before 70 years in 112 of 183 countries. The total incidence of cancer in 2020 is estimated to be around 19.292.789 cases globally and 396.914 cases in Indonesia. The type of cancer with the highest incidence worldwide is breast cancer. (Sung et al., 2021)

Traditional cancer therapies include surgery, chemotherapy, and radiation. Chemotherapy is the administration of cytotoxic agents that can cause cell damage and kill cancer cells. Chemotherapy using a cytotoxic agent can effectively treat cancer, especially in combined with other therapeutic methods such as surgery and radiotherapy or as single treatment such as in leukemia treatment. Patient condition, stage and type of cancer, and chemotherapy regimen can affect the treatment effectiveness (Jayachandran et al., 2014; McArthur et al., 2011). The delivery system is essential to determine the success of chemotherapy. Drug delivery based on nanomedicine or nanotechnology is effective for cancer treatment because it can inhibit cancer cells compared to drugs without modification (Tran et al., 2017). Nanotechnology products can be liposomes, nanocrystal, solid lipid nanoparticles, nanoencapsulation, metal nanoparticles, nanoemulsion, nanosuspension, polymeric micelles, and dendrimers. Each dosage form has its characteristics, advantages, and disadvantages (Park, 2007).

Nanoencapsulation is defined as a technology to encapsulate substances by film or polymeric material at the nanoscale range (Suganya & Anuradha, 2017). The nanoencapsulation produces nanocapsules, a vesicular system of the bioactive compound surrounded by a polymer membrane with particle size 10-1000 nm. The nanoencapsulation process can enhance drug bioavailability, improve the release and targeting of drugs. This process also can maintain the stability of the active substance in the presence of a coating polymer. Due to its various advantages, nanoencapsulation is widely applied in drug development, including cytotoxic agents. One of the critical factors that influence the successful development of nanocapsules systems is a selection of coating polymer. The polymer must form good coating properties and improve the characteristics of the active compounds according to the intended use. (Karnakar et al., 2021). This research was conducted to study the development of nanocapsules containing cytotoxic agents. This study focused on determining the type of polymers for nanoencapsulation of cytotoxic agents and analyze the effect of nanoencapsulation on the cytotoxic activity.

METHODS

The research was conducted with a systematic literature review (SLR), with several stages: searching, filtering, final inclusion, and data extraction. Article obtained from the reputable database Science Direct, Taylor and Francis, PubMed, dan Sage Publication. The keywords used for article search are "nanocapsules," "nanoencapsulation," "polymeric nanoparticles," "cancer," "cytotoxic". From the literature searching stage, 606 articles were obtained (391 from Science Direct, 31 from PubMed, 100 from Taylor and Francis, 84 from Sage Publications). The articles further selection referring to inclusion and exclusion criteria. The inclusion criteria is an article on nanocapsules for a cytotoxic agent published in the last ten years. The exclusion criteria in this study are review article, nanocapsules article as a cytotoxic without an activity test, full text can't be assessed. Twelve selected papers met the inclusion and exclusion criteria (Six articles from PubMed, five from ScienceDirect, and one from sage Publications). Data about the characteristic of an active compound, type of polymer, nanocapsules characteristics, and cytotoxic activity was extracted from each article that meets the inclusion and exclusion criteria.

RESULTS AND DISCUSSION

The nanoencapsulation process was widely used in the pharmaceuticals field, including for cytotoxic agents. Nanocapsules are submicroscopic colloidal drug carrier systems in which the drug is confined to a cavity consisting of an inner core surrounded by a polymeric membrane (figure 1). Many kinds of polymer can be used for nanocapsule development, including natural and synthetic polymers

(Dineshkumar et al., 2013). In general, the polymer for nanoencapsulation must form a coating film with desired properties in the nanoscale range (Suganya & Anuradha, 2017). In table 1, was described the type of polymers were used for cytotoxic nanocapsules development. The characteristics of the nanocapsules were also presented, including particle size, polydispersity index, zeta potential, and entrapment efficiency.

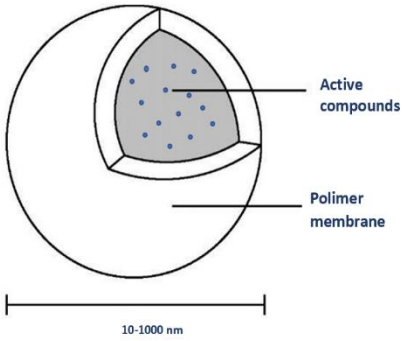

Figure 1. Nanocapsules structure

One of the polymers is widely used to develop cytotoxic nanocapsules, according to table 1, is PLGA (poly (lactic-co-glycolic acid)). PLGA was used in grandisin and polyalthic acid nanoencapsulation. PLGA is a synthetic copolymer that has been used extensively for drug delivery due to its biodegradable and biocompatible nature (Teixeira et al., 2005). PLGA is convenient as a polymer for nanoencapsulation resulting in appropriate membrane characteristics (Anversa Dimer et al., 2020). PLGA is a family of Food Drug Administration (FDA)-approved polymers for drug development. (Anversa Dimer et al., 2020).

At pomegranate extract, punicalagin, ellagic acid, and honociol nanocapsules, PLGA as a polymer was modified, being PEGylated PLGA (PLGA-PEG). Compare with PLGA, PEGylated PLGA increases hydrophilicity, enhances blood circulation time, and improves pharmacokinetics by preventing opsonization and uptake by the mononuclear in cancer treatment. PEGylation also could improve the pharmacokinetic properties related to increasing biological half-life and bioavailability. (Rezvantalab et al., 2018)

Another polymer for the development of cytotoxic nanocapsules is polycaprolactone (PCL). PCL was used for nanoencapsulation of pentacyclic triterpene, lapatinib, and erlotinib. PCL is a biocompatible and biodegradable polymer with a semi-crystalline nature. The unique advantages of PCL are elasticity properties, mechanical stability, safety, biocompatibility, and prolonged degradation. The FDA approved PCL in the USA for biomedical uses. (A et al., 2015; Elhesaisy & Swidan, 2020).

Polymers were used for nanoencapsulation of *Gymnema sylvestre* extract, and lectin is combination chitosan and sodium tripolyphosphate. Chitosan is a natural cationic polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as shrimps, lobster, and crabs. The advantages of the application of chitosan are biocompatibility, biodegradability, and low immunogenicity properties. Sodium tripolyphosphate (TPP) is a non-toxic chemical crosslinking agent. Sodium TPP has a high negative charge that could interact with polycationic chitosan through polyionic interaction (ionic gelation method). Sodium TPP can strengthen the matrix of chitosan.(Bangun et al., 2018; Riezk et al., 2020).

In docetaxel nanocapsules, chitosan was used as a polymer in combination with sodium alginate. Similar to the chitosan-Sodium TPP, a combination of chitosan and sodium alginate forming polyionic hydrogels. The ammonium groups of chitosan and the carboxylate groups of alginates interact through ionic interaction. The characteristics of this polymer combination are biocompatible, biodegradable, non-toxic, and provide sustained release of encapsulated materials. (Nagarwal et al., 2012)

In camptothecin nanocapsules, heparin and poly(l-lysine)/PLL were used as nanocoating polymers. Heparin is a biocompatible material that can be obtained naturally and synthetically. Heparin is an anionic polysaccharide that is composed of repeating glucosamine and uronic acid residues. PLL is a cationic polymer of amino acids. Combination heparin and PLL, forming polyionic complexes in the nanoencapsulation process. (Na et al., 2007; Rodriguez-Torres et al., 2018)

Table 1. Development of cytotoxic nanocapsules

Active substances	**Polymer**	**Method**	**Nanocapsules characteristic**	**References**
Grandisin	PLGA	Nanoprecipitation (interfacial deposition)	PS : 169.5±0.28 nm PDI : 0.141±0.004 ZP : -10.82±0.43 mV EE : 98.20±0.65%	(Stecanella et al., 2013)
Polyalthic Acid	PLGA	Nanoprecipitation	PS: 98.64 ± 28 nm PDI : 0.057 ZP: −8.46 ± 1.22 V EE :98 ± 0.005%	(Chibas et al., 2019)
Pomegranate extract	PLGA-PEG	Double emulsion–solvent evaporation	PS : 160±5 nm PDI: 0,084±0,04 ZP: -13±1,53 mV	(Reliene et al., 2015)
Punicalagin	PLGA-PEG	Double emulsion–solvent evaporation	PS: 216±3 nm PDI: 0.16+0.04 ZP:-13.3±1.84 mV	(Reliene et al., 2015)
Ellagic acid (EA)	PLGA-PEG	Double emulsion–solvent evaporation	PS: 175±3 nm PDI: 0.14±0.03 ZP: -6.06±0.5 mV	(Reliene et al., 2015)
Honokiol	PLGA-PEG	Nanoprecipitation	PS: 125±11.51 nm PDI: 0.19±0.06	(Makadia & Siegel, 2011)

			ZP : -6.21±0.97 mV EE: 94.18±1.82%	
Pentacyclic triterpene	Polycaprolactone (PCL)	Nanoprecipitation	PS: 122.7 ± 2.06 nm PDI: 0.124 ± 0.010 ZP : −7.12 ± 0.35 mV	(Silva-Filho et al., 2020)
Lapatinib	Polycaprolactone (PCL)	Interfacial deposition	PS : 172 ± 8 nm PDI : 0.100 ± 0.012 ZP: −8.85 ± 1.76 mV EE : 98.77 ± 2.01%	(Buss et al., 2019)
Erlotinib	Polycaprolactone (PCL)	Interfacial deposition	PS : 171 ± 2 nm PDI: 0.076 ± 0.015 ZP : − 8.38 ± 1.06 mV EE : 97.37 ± 2.72%	(Bruinsmann et al., 2020)
Gymnema sylvestre extract	Chitosan -sodium tripolyphosphate (TPP)	Ionic gelation	PS: 58-80 nm PDI: 0,474 ZP : +3.25 mV	(Anbu A et al., 2016)
Lectin (From *Lepidium sativum*)	Chitosan -sodium tripolyphosphate (TPP)	Ionic gelation	PS: 298.10 ± 1.9 nm, ZP: 21.05 ± 0.95 mv PDI : 0.468 EE: 52:435 ± 0:09%	(Yasin et al., 2020)
Docetaxel	Chitosan-sodium alginate	Layer by layer	PS : 354.4±12.98 nm PDI: 0.193±0.014 ZP: +34.64± 1.5 mV EE: 94.45±2.54%	(Singh et al., 2015)
Camptothecin	Heparin-PEGylated polylysine	Layer by layer	PS: 123 ± 2 nm	(Parekh et al., 2014)
Nitrochalcone	Folic acid-PMMA (polymethyl methacrylate)	Miniemulsion polymerization	PS : 170 ± 6 nm ZP : −40 ± 4 mV EE: 80± 5%	(Carpio Arévalo et al., 2019)

PMMA (polymethyl methacrylate) was used in the development of nitrochalcone nanocapsules. PMMA is widely used for nanoencapsulation due to its biocompatibility, chemical stability, nontoxicity, and good mechanical properties. PMMA was linked with folic acid for providing targeted drug delivery for the cancer cell. Folic acid (vitamin B9) can increase cell uptake since folate receptors are overexpressed in many human cancer cells. (Carpio Arévalo et al., 2019; Yu et al., 2015)

Based on the discussion above, it is known that several types of polymers can be used for the development of nanocapsules, especially for cytotoxic agents. Those polymers are PLGA, PEGylated PLGA, PCL, chitosan-Sodium TPP, chitosan-sodium alginate, Heparin-PLL, and PMMA. Those polymers are widely used for nanocapsules formulation due to their biocompatible, biodegradable, non-toxic, capable of forming a film, and providing the desired coating properties. The polymer that was used was compatible with the active agent at the core of nanocapsules. The study about the correlation between active agent characteristics and polymer selection must be further analyzed. Those polymers were used for the nanoencapsulation process using various methods, including nanoprecipitation, interfacial deposition, double emulsion–solvent evaporation, ionic gelation, layer by layer, and miniemulsion polymerization.

Cytotoxic nanocapsules in table 1 have good characteristics according to particle size, PDI, and entrapment efficiency. The particle size of cytotoxic nanocapsules varied between 58-354 nm with PDI is 0.057-0.474. Those results meet the requirement of particle size (<1000 nm) and PDI (<0.5). In contrast with particle size and PDI, some nanocapsules do not meet potential zeta requirements (>±30 mV). Zeta potential is essential to control the stability of a nanoparticle. Further study must be conducted to ensure the storage stability of the preparation with zeta potential <±30 mV. Another characteristic that is essential in nanocapsules formulation is entrapment efficiency. In general, the entrapment efficiency of cytotoxic nanocapsules was appropriate (>80%) except at lectin nanocapsules using chitosan-sodium TPP. Further optimization on lectin formulation must be conducted to improve its entrapment efficiency.

Table 2 shows the effect of nanocapsules formulation for cytotoxic activity of the active compounds. The cytotoxic activity test was conducted in vitro, commonly using The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method. This section will explain in detail, the impact of the nanoencapsulation process on the activity of each compound.

Grandisin is a phytoconstituent isolated from *Virola surinamensis,* demonstrating antitumoral activity. Grandisin was developed as being a nanocapsules system due to low water solubility and high lipophilicity properties. Nanoencapsulation on grandisin could increase its cytotoxic activity, marked by decreased IC_{50} from 0.078 µM in pure drug to 0.005 µM in nanocapsules. That's due to a higher cellular uptake and drug accumulation in tumor tissue. (Stecanella et al., 2013)

Polyalthic acid is a diterpene of the *Copaifera* genus. Polyalthic acid shows antitumor activity but poor water solubility. The pharmaceutical modification must be conducted to improve its pharmacological activity. The nanoencapsulation of polyalthic acid enhances its cytotoxic effects on MCF-7 breast cancer cells, indicated by decreasing IC_{50} value 2.8-fold. The reduction of particle size and the negative charge of surface particles impact cytotoxic activity. (Chibas et al., 2019)

Tabel 2. Data of In-vitro Cytotoxic Activity Test

Active Compounds	Type of cancer cell	Inhibitory Concentration 50% (IC_{50})			References
		Pure drug (PD)	Nanocapsules (NC)	Ratio (PD/NC)	
Grandisin	3T3-A31/fibroblasts	0.078 µM	0.005 µM	15.6	(Stecanella et al., 2013)
Polyalthic Acid	MCF-7 /breast	751 µM	270.3 µM	2.8	(Chibas et al., 2019)
	MCF-7/breast	44.34 µg/mL	19.36 µg/mL	2.3	

Pomegranate extract	Hs578T/breast	61.93 µg/mL	29.17 µg/mL	2.1	(Reliene et al., 2015)
Punicalagin	MCF-7/breast	44.42 µg/mL	8.13 µg/mL	5.5	(Reliene et al., 2015)
	Hs578T/breast	52.57 µg/mL	4.45 µg/mL	12.5	
Ellagic acid (EA)	MCF-7/breast	35.93 µg/mL	15.22 µg/mL	2.4	(Reliene et al., 2015)
	Hs578T/breast	59.94 µg/mL	25.15 µg/mL	2.4	
Honokiol	MCF-7/breast	52.63 ± 5.4 µM	20 ± 2.3 µM	2.6	(Makadia & Siegel, 2011)
	EAC/breast	27.7 µM	10 µM	2.7	
Pentacyclic triterpene	HCT116/colon	1.07 µg/mL	0.26 µg/mL	4.1	(Silva-Filho et al., 2020)
	PC3/prostate	1.2 µg/mL	0.15 µg/mL	8.0	
	SNB19/glioblastoma	1.44 µg/mL	0.11 µg/mL	9.6	
Lapatinib	T24/bladder	17.2 µM	5.1 µM	3.4	(Buss et al., 2019)
Erlotinib	A534/lung	14.45 µM	0.94 µM	1.4	(Bruinsmann et al., 2020)
G. sylvestre extract	PA01/ovarium	931 µg/mL	378.4 µg/mL	2.5	(Anbu A et al., 2016)
Lectin	HepG2/liver	265 µg/mL	105 µg/mL	2.5	(Yasin et al., 2020)
Docetaxel	MCF-7/breast	20 nM	5 nM	4.0	(Singh et al., 2015)
Camptothecin	CRL2303/ glioblastoma	143.6 nM	103.8 nM	1.4	(Parekh et al., 2014)
Nitrochalcone	Hela cells/cervic	66.9 µM.	46.7 µM	1.4	(Carpio Arévalo et al., 2019)

Pomegranate extract exhibits anticancer effects in vitro and in vivo due to the content of ellagitannins. Pomegranate extract has antiproliferative and pro-apoptotic activities. Ellagitannins on pomegranate extract show unstable properties due to susceptibility to enzymatic hydrolysis. The most abundant ellagitannin in pomegranates is punicalagin. Pomegranate extract, punicalagin, and another glycoside isolate of pomegranate (ellagic acid) were nano encapsulated to improve their bioavailability and chemical stability. The results show that the nanoencapsulation process increased the cytotoxic activity of pomegranate extract, punicalagin, and ellagic acid on MCF-7 breast cancer cells 2.3, 5.5, and 2.4 folds compare with pure drugs, respectively. At the same time, the activity against Hs578T breast cancer cells increases 2.1, 12.5, and 2.4 folds, respectively. The nanocapsules system could protect the pomegranate's bioactive compounds from rapid hydrolysis and provide sustained release behavior into the cell. PLGA nanoparticles are taken up by cells via fluid-phase pinocytosis or clathrin-mediated endocytosis. (Reliene et al., 2015)

Honokiol is a bioactive polyphenol extracted from Magnolia species, herbal medicine in China and Japan. It possesses many pharmacological activities, including as an anticancer. That compound shows poor water solubility properties and limited its therapeutic applicability. Nanoencapsulation of honokiol succeed in increasing cytotoxic activity on MCF-7 and EAC breast cancer cell lines. Nanocapsules system could deliver honokiol into the subcellular site of action meanwhile preserving its anticancer activity after formulation. (Stecanella et al., 2013)

The pentacyclic triterpene 3β,6β,16β-tri-hydroxilup-20(29)-ene is a very abundant secondary metabolite of *C. leprosum* leaves. That compound shows cytotoxic activities in adenocarcinoma, hepatoma, bladder cancer, and colorectal carcinoma. The limiting problems for developing that compound for pharmaceutical application are instability towards heat, oxidation, light and low solubility. Nanoencapsulation was selected to improve the cytotoxic activity. The result shows that the cytotoxic activities of encapsulated compounds increased 4.1-9.6 folds in 3 types of cancer cells compared with pure drugs. (Silva-Filho et al., 2020)

Lapatinib is a tyrosine kinase inhibitor, approved by the FDA to treat advanced or metastatic breast cancer in 2007. Lapatinib presents low water solubility and bioavailability. Nanoencapsulation of lapatinib known could decrease the IC_{50} value in T24 bladder cancer cell line 3.4-fold. Nanocapsules of lapatinib inducing cell cycle arrest and apoptosis exhibiting better effects compared to the non-encapsulated form. (Buss et al., 2019)

Erlotinib acts as a reversible competitive inhibitor of ATP that binds to the endothelial growth factor receptor (EGFR). FDA approved erlotinib for the treatment of locally advanced or metastatic lung cancer. Erlotinib is categorized into BCS class II due to low water solubility and high permeability. Nanoencapsulation of this compound significantly increases cytotoxic activity to A534 lung cancer cell line, 15.4 fold compare with pure drug (Bruinsmann et al., 2020). Lapatinib and erlotinib using PCS as coating polymer. PCS could enhance the cellular uptake of a drug through the cancer cell membrane. (Korang-Yeboah et al., 2015)

Gymnema sylvestre R. extract containing many bioactive substances possesses many pharmacological effects, including anticancer. Nanoencapsulation of this extract using chitosan increasing cytotoxicity to PA01 ovarium cancer cell (2.5 folds). The Nanocapsules system increases the cellular uptake of bioactive compounds into the cancer cell. The cellular uptake of nanoparticles was facilitated by the chitosan's positive surface charge and interactions with biomolecules complex. (Anbu A et al., 2016)

Lectins are the oligomeric sugar-specific glycoprotein that involved many physiological functions, including antifungal, antiviral, antitumor, and cell agglutination. Chitosan-sodium TPP was used to encapsulate lectin. The encapsulation process improves the cytotoxicity of lectin to the HepG2 liver cancer cell line. Chitosan nanoparticles are inheriting more stability to protein's shelf-life and providing better permeability. (Yasin et al., 2020)

Docetaxel is an inhibitor of microtubule depolymerization with significant activity against breast cancer. Low solubility and metabolic degradation are a problem for the formulation of docetaxel. Modification of docetaxel into nanocapsules system improves its cytotoxicity (4 folds). The increase of cellular drug uptake affects its cytotoxicity. The main factor influencing the cellular uptake is the positive charge on nano reservoirs in docetaxel nanocapsules. The cell membrane possesses large negatively charged domains, which should interact with the nanoparticle membrane. (Singh et al., 2015)

Camptothecin, isolate from *Camptotheca acuminata* bark/stem, is a cytotoxic topoisomerase I-specific quinoline alkaloid inhibitor. Camptothecin developed to nanocapsules system due to its low water solubility with low stability. Camptothecin easily hydrolyses into carboxylate already at neutral and slightly alkaline pH. Nanoencapsulation of camptothecin increase is cytotoxicity to CRL2303 glioblastoma cancer cell line. The encapsulation of CPT in polyelectrolytes membrane reduces the rate of hydrolysis of lactone compared with free drug, resulting in enhancement of activity toward glioblastoma cells. (Parekh et al., 2014)

4-nitrochalcone, derivate chalcone, is metabolic intermediates in the flavonoid synthetic pathway, with cytotoxic effects on tumor cells. 4-nitrochalcone was encapsulated by folic acid-PMMA and increase its cytotoxicity towards Hela cervical cancer lines. The nanoencapsulation process improves the cellular uptake of drugs through the receptor-mediated endocytosis pathway. (Parekh et al., 2014)

Based on table 2, many cytotoxic agents have been developed to be nanocapsules systems. The problem in drug characteristics, including low water solubility, chemical instability, and low bioavailability, is a reason for conducting nanoencapsulation for a cytotoxic agent. Based on data from table 2, we can conclude that the nanoencapsulation on cytotoxic agents significantly increases the activity marked by the decrease of IC_{50} value in the range 1.4-15.4 folds, compare with pure drugs. Several factors cause increased cytotoxic activity in the nanocapsule system. Nanocapsules can facilitate cell membrane diffusion due to their nano size, which is appropriate with a pore size of the cancer cell is 300-700 nm. Encapsulation of bioactive compounds by coating polymer causing surface particle modification that enhances cellular uptake of drugs related to a specific interaction between a polymer and cell membrane.

The nanoencapsulation can maintain the stability of the active substance, so it has better activity than pure drugs.

CONCLUSION

Many cytotoxic agents have been developed in nanocapsules systems due to their low water solubility, chemical instability, and low bioavailability properties. Synthetic or natural polymers applied for cytotoxic nanocapsules are PLGA, PEGylated PLGA, PCL, chitosan-sodium TPP, chitosan-sodium alginate, heparin-PLL, and PMMA. Those polymers are widely used for nanocapsules formulation related to their biocompatibility, biodegradable, non-toxic, and providing the desired coating properties. The nanoencapsulation on cytotoxic agents significantly increases the in vitro cytotoxicity marked by the decrease of IC_{50} value in the range 1.4-15.4 folds compare with pure drugs. The increase in cytotoxicity is caused by particle size reduction, modification of particle surface properties, and enhancement of drug stability.

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