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Andrographolide Loaded in Micro- and Nano-Formulations: Improved Bioavailability, Target-Tissue Distribution, and Efficacy of the "King of Bitters"

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ABSTRACT

Andrographolide (AG) is the characteristic constituent of *Andrographis paniculata*, of the Acanthaceae family. This plant is a well-known Asian medicinal plant that is widely used in India, China, and Thailand. A monograph of *Herba Andrographidis* (Chuanxinlian) is included in the *Chinese Pharmacopoeia*, which reports that this decoction can "remove heat, counteract toxicity, and reduce swellings." The numerous potential activities of AG range from anti-inflammatory to anti-diabetic action, from neuroprotection to antitumor activity, and from hepatoprotective to anti-obesity properties. However, AG has low bioavailability and poor water solubility, which can limit its distribution and accumulation in the body after administration. In addition, AG is not stable in gastrointestinal alkaline and acidic environments, and has been reported to have a very short half-life. Among the diverse strategies that have been adopted to increase AG water solubility and permeability, the technological approach is the most useful way to develop appropriate delivery systems. This review reports on published studies related to microparticles (MPs) and nanoparticles (NPs) loaded with AG. MPs based on polylactic-glycolic acid (PLGA), alginic acid, and glucan derivatives have been developed for parenteral oral and pulmonary administration, respectively. NPs include vesicles (both liposomes and niosomes); polymeric NPs (based on polyvinyl alcohol, polymerized phenylboronic acid, PLGA, human serum albumin, poly ethylcyanoacrylate, and polymeric micelles); solid lipid NPs; microemulsions and nanoemulsions; gold NPs; nanocrystals; and nanosuspensions. Improved bioavailability, target-tissue distribution, and efficacy of AG loaded in the described drug delivery systems have been reported.

1. Introduction

Andrographis paniculata (Burm.f.) Nees is a well-known Asian medicinal plant of the Acanthaceae family (Fig. 1). It is called Fa-Tha-Lai-Jone in Thailand [1]; Kalmegh in Ayurvedic medicine; and Chiretta or "king of bitters" in India, China, and Thailand [2,3]. A monograph of *Herba Andrographidis* (Chuanxinlian) is included in the *Chinese Pharmacopoeia*, which reports that this decoction acts to "remove heat, counteract toxicity, and reduce swellings" [4]. Traditional indications for the use of this decoction include effects in the upper respiratory tract, such as the common cold, bronchitis, sinusitis, pharyngotonsillitis, whooping cough, pneumonia, and otitis media. In addition, activity against diarrhea, enteritis, lower urinary infections, dermatitis, and tuberculosis has traditionally been reported [4–6].

Activity against the common cold, pharyngotonsillitis, uncomplicated sinusitis, bronchitis, acute diarrhea, and urinary infections is also supported by clinical data reported in the World Health Organization (WHO) monograph on *Herba Andrographidis* [7].

The main active constituents of *Herba Andrographidis* are bicyclic diterpenes with a γ-lactone moiety, which principally include andrographolide (AG, Fig. 2) and its analogs, 14-deoxyandrographolide, neoandrographolide, and 14-deoxy-11-12 -didehydroandrographolide [8]. In addition to the numerous potential activities of AG, which range from anti-inflammatory to anti-diabetic action, from neuroprotection to antitumor activity, and from hepatoprotective to anti-obesity properties [9], AG has poor water solubility, which limits its distribution and

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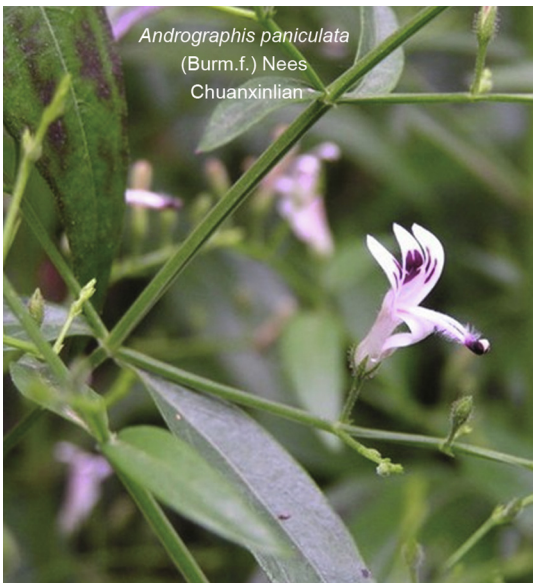

Fig. 1. *Andrographis paniculata* (Burm.f.) Nees, Acanthaceae.

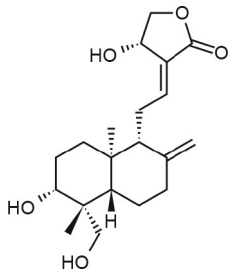

Fig. 2. Andrographolide (AG), the main characteristic diterpenoid in *Andrographis paniculata*.

accumulation in the body after administration. In addition, AG is unstable in gastrointestinal media and possesses a very limited half-life (circa (ca.) 2 h) [10].

2. Microparticles and nanoparticles loaded with AG

AG is a very promising natural product with various potential therapeutic benefits; however, it has not reached its milestone therapeutic potential due to its low bioavailability when administered in conventional dosage forms. The development of suitable delivery systems for AG is an urgent issue in the development of efficacious therapeutic approaches for this compound. Among the diverse strategies that have been adopted to increase AG water solubility and permeability, the technological approach is the most useful way to develop suitable delivery systems. A very simple approach to improve the solubility of a molecule is to modify the particle size through micronization and nanonization techniques, which can enhance the total surface area with a consequent improvement of the dissolution behavior. Microparticles (MPs)—solids or small droplets of liquids enclosed in natural or synthetic polymers of variable thickness and permeability—are frequently used in this endeavor in order to obtain a controlled release of the encapsulated drug. Both organic and inorganic nanoplatforms (Fig. 3) can also increase solubility to an extraordinary degree, and can enhance photostability, chemical stability, bioavailability, and tissue distribution. These nanocarriers can overcome multidrug-resistance phenomena and cross biological barriers, including the blood–brain barrier (BBB). Organic nanoparticles (NPs) include polymeric and lipid NPs. Among lipid NPs, vesicles, microemulsions (MEs), nanoemulsions (NEs), and solid lipid NPs (SLNs) are the most widely investigated. Finally, the simple use of nanopowders ranging in size from 10 to 1000 nm are an alternative way to enhance the solubility of AG, principally due to the increased dissolution properties that result from the greater surface areas in comparison with similar masses of larger scale materials. For these formulations, which are mainly suitable for oral or parenteral administration, surfactants or hydrophilic polymers should be added during milling for surface stabilization, in order to inhibit the formation of aggregates.

2.1. Microparticles

MPs include microspheres and microcapsules. A microcapsule is characterized by an internal core and an external shell. The internal core can be liquid (i.e., oil or water) or solid, and typically contains the active ingredient, while the shell is usually a polymer or wax. A microsphere is a solid matrix particle; the active ingredient is usually dissolved or melted in the matrix.

A study investigated the development of sustained-release microspheres based on the copolymer obtained from lactic and glycolic acid—polylactic-glycolic acid (PLGA)—loaded with AG. PLGA microspheres were prepared by an emulsion solvent evaporation method. The formulation was optimized using response surface methodology, which was used to identify the optimum levels of the process variables, which had significant effects on particle size and entrapment efficiency. The study focused on the development of spherical microspheres with an average particle size of (53.18 ± 2.11) μm. The entrapment efficiency (EE) was 75.79% ± 3.02%, while the drug loading was 47.06% ± 2.18%. The release kinetics followed the Korsmeyer-Peppas model, with a low initial burst followed by a successive prolonged release (up to 9 d). After intramuscular administration of the MPs, AG plasma concentrations were found to be moderately high over a period of one week [11].

It is generally accepted that high doses of AG provide hepatic protection; however, due to the extremely bitter properties of AG, sickness and nausea can frequently occur. To address this issue, bitterless MPs prepared with sodium alginate and calcium ions were developed using various AG:alginate ratios (i.e., 1:2, 1:1, and 2:1). The release kinetics of the developed MPs fitted well with the Korsmeyer-Peppas equation: The MPs released at acidic pH (1.2 or 4.0) with ca. 15% release for up to 4 h; a complete release of the remaining AG then occurred at pH 7.4. The developed MPs were very useful for the oral delivery of AG and had a good EE, with 86% release [12]. In a further study, the same authors investigated the mechanism of gelation for the entrapment of AG. AG stability was obtained in the cross-linked MPs [13]. A micronized formulation for a dry powder inhaler (DPI) was also developed using a natural polysaccharide, scleroglucan. The MPs displayed a mean aerodynamic diameter of (3.37 ± 0.47) μm. The *in vivo* studies exhibited a suitable AG lung deposition, and no inflammation or toxicity was found after 24 h. Moreover, the formulation demonstrated improved activity in pulmonary arterial hypertension [14].

2.2. Nanoparticles

2.2.1. Polymeric nanoparticles

Polymeric nanoparticles (PNPs, Fig. 3) are the most widespread delivery systems. These include nanospheres and nanocapsules. Nanospheres are matrix systems in which the drug is homogeneously distributed, while in nanocapsules, the drug is contained in a core, which is walled in by an outer shell. Polymers used for the preparation of PNPs are classified as synthetic or natural (i.e.,

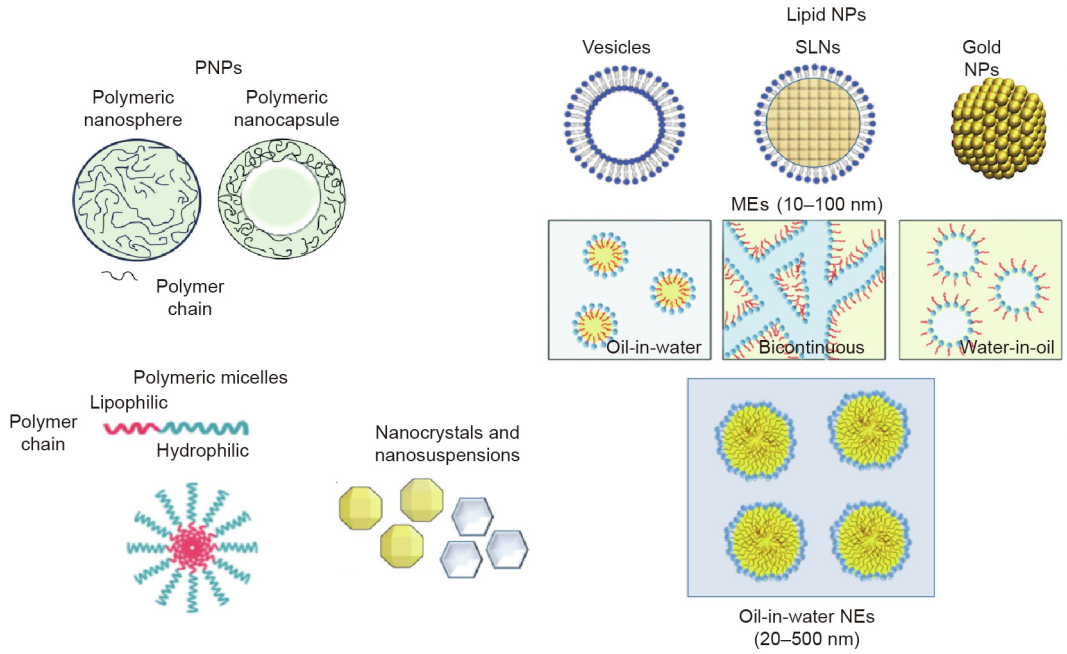

Fig. 3. Nanocarriers have been developed to improve the bioavailability, target-tissue distribution, and efficacy of AG, the "king of bitters." NPs: nanoparticles; PNPs: polymeric nanoparticles; SLNs: solid lipid NPs; MEs: microemulsions; NEs: nanoemulsions.

biopolymers), and are commonly classified as biodegradable or non-biodegradable as well. Biodegradation of a polymer involves the cleavage of hydrolytic or enzymatic bonds in the polymer, leading to polymer erosion. Most naturally occurring polymers undergo enzymatic degradation and are thus the first biodegradable biomaterials used in pharmaceutical technology. Natural polymers include proteins (e.g., albumin, gelatin, soy protein hydrolysate, and casein) and polysaccharides (pectin, cellulose, starch, gum arabic, carrageenan, alginate, xanthan gum, gellan gum, and chitosan) [15].

Roy et al. [16] developed PLGA PNPs loaded with AG and coated with chitosan for antitumor therapy. The PNPs were nontoxic and increased the anticancer activity by threefold in the MCF-7 cell line and *in vivo* studies using Ehrlich ascites carcinoma (EAC) cells, in comparison with unformulated AG. The anticancer activity of AG loaded in other PNPs was tested for specific targets. Kim et al. [17] formulated water-soluble PNPs for systemic administration, using a polymer obtained from phenylboronic acid. This nanocarrier displayed exceptional targeting properties both *in vitro* and *in vivo*, and resulted in a significant decrease of *in vivo* tumor growth. Roy et al. [18] prepared AG loaded in PLGA PNPs (50:50), which were stabilized with polyvinyl alcohol (PVA). The study focused on AG delivery into macrophage cells infected with leishmanial parasite. The PNP average size was 173 nm, with a surface charge of −34.8 mV. Activity on the infested macrophages was significant in the case of PNPs containing 4% PVA (IC_{50} of 34 μmol·L^{-1}), which is a one-quarter dose of pure AG (IC_{50} of 160 μmol·L^{-1}). The same authors recently prepared PNPs with hepatoprotective activity for use in hepatotoxic conditions. Cationic modified PLGA PNPs loaded with AG were developed, and showed superior dissolution behavior in comparison with pure AG and favorable cytokine regulation in the hepatic tissues, leading to a rapid recovery of mouse liver [19]. Moreover, in 2017, Das et al. [20] explored the effectiveness of AG encapsulated in PLGA PNPs against liver damage in mice induced by arsenic. The PNPs had an average diameter of 65.8 nm and the EE was 64%. The NPs increased the level of reduced glutathione and antioxidant enzymes, including superoxide dismutase and catalase. The protective efficiency of AG loaded in PNPs was about five times greater in comparison with unformulated AG. NP administration improved the liver tissue architecture, suggesting a beneficial effect against arsenic-induced liver toxicity. AG has also been reported to ameliorate neurodegenerative disorders. To overcome its low brain distribution, Guccione et al. [10] loaded AG into albumin NPs (ANPs) and ethylcyanoacrylate NPs (ENPs). The ability of both NPs to permeate the BBB was investigated using an *in vitro* BBB model, hCMEC/D3. Although AG was unable to cross the BBB model to any significant degree, the ANPs improved the permeation of AG by twofold. In addition, the integrity of the cell layer was maintained. In contrast, while ENPs increased the cross properties of AG, the BBB was temporarily disordered. Another nanovector that was tested involved pH-sensitive PNPs based on a cationic poly methacrylate copolymer (Eudragit® EPO). The optimized formulation consisted of Pluronic® F-68 (0.6%, w/v) and Eudragit® EPO (0.45%, w/v). It had a very high EE (93.8% ± 0.67%), homogeneous particle size ((255 ± 9) nm), and good superficial charge values ((29.3 ± 3.4) mV). The *in vivo* absorption of AG and of AG loaded in the NPs was studied at a dose of 10 mg·kg^{-1} in male Wistar albino rats. The AG loaded in PNPs caused a tremendous increase in area under the curve ($AUC_{0-\infty}$) (ca. 2.2 fold) and the maximum concentration (C_{max}) (3.2 fold) in comparison with unformulated AG. In addition, the relative bioavailability increased by 121.53% ($P < 0.05$) in comparison with pure AG. Other parameters were favorably affected by the loading of AG in NPs: A smaller amount of time that AG is present at the maximum concentration in serum (T_{max}) (4.0 fold) and a reduction in Cl/F (2.2 fold) were observed [21].

2.2.2. Polymeric micelles

Polymeric micelles (Fig. 3) are self-assembling colloids of amphiphilic polymers, which can spontaneously aggregate in a particular solvent (generally water). AG was entrapped in a micellar formulation based on an amphiphilic triblock copolymer of *D,L*-lactic acid, glycolic acid, and ethylene glycol(poly(lactide-*co*-glycolide)-*block*-poly(ethylene glycol)-*block*-poly(lactide-*co*-glycolide) (PLGA-PEG-PLGA)), and then evaluated for bioavailability *in vivo* and for *in vitro* cytotoxicity. Cellular uptake and

cytotoxicity, including cell cycle arrest, proliferation inhibition, and pro-apoptosis effects were tested against human breast cancer MAD-MD-231 cells. The loading efficiency of the micelles was about 92%, and the particle size was (124.3 ± 6.4) nm. The micelles exhibited a higher inhibition of proliferation when compared with the unformulated AG. The highest effectiveness of cellular uptake and intracellular transport, pro-apoptotic properties, and cell cycle arrest at the G_2/M phase were found in MAD-MD-231 cells using the micelles. Pharmacokinetics studies were carried out in rats, and both the mean residence time and plasma $AUC_{0-\infty}$ were found to increase by almost threefold in comparison with unformulated AG [22].

In a further study, a series of copolymers obtained from methoxy poly(ethylene glycol)-poly(*D,L*-lactic acid) (mPEG-PLA) with various ratios of hydrophilic to hydrophobic portions was synthetized to encapsulate AG. The micelles had a size of (92.84 ± 5.63) nm, a high EE of 91.00% ± 11.53%, and a loading capacity of 32.14% ± 3.02% (w/w). mPEG-PLA loaded with AG was found to have good stability against salt dissociation, protein adsorption, and anion substitution. The solubility of AG and a derivative of AG (14-deoxy-11,12-didehydroandrographolide) in micelles increased by 4.51 times and 2.12 times in water in comparison with unformulated AG. mPEG-PLA loaded with AG showed the same release profile in a different dissolution medium. Cytotoxicity testing *in vitro* demonstrated that AG loaded in mPEG-PLA exhibited higher cell viability inhibition in mouse breast cancer 4T1 than free AG [23].

2.2.3. Vesicles

Vesicles (Fig. 3) are colloidal vectors formed by bilayers, which can load hydrophilic and hydrophobic compounds. Liposomes are principally constituted of natural phospholipids and cholesterol, while niosomes are nonionic surfactant-based vesicles [24].

A very recent study by Kang et al. [25] reported on liposomal co-delivery of AG and doxorubicin to inhibit breast cancer growth and metastasis. The liposome was prepared with a cell-penetrating peptide, which was able to inhibit the *in vitro* proliferation of 4T1 cells. Two distinctive tests were done: a wound-healing assay and a trans well invasion assay. The liposome was found to enhance AG accumulation in tumors and to result in high intra-tumor penetration in a tumor mouse model of breast. A synergistic effect of doxorubicin and AG was found. In another study, AG-loaded niosomes were prepared. The niosomes improved the tissue distribution and bioavailability of AG in mice. In particular, AG-loaded niosomes accumulated in the liver much more than the free drug. *In vitro* studies on anti-hepatocellular carcinoma (HCC) efficacy in HepG2 cells disclosed no significant differences between the free drug and AG-loaded niosomes [26]. In a further study, loading AG in a natural soya-phosphatidylcholine mixture was found to enhance the absorption and hepatoprotective activity of AG in comparison with unformulated AG. The study proved the hepatoprotective potential of AG using a rat model of hepatotoxicity induced by carbon tetrachloride. The results showed significantly increased absorption, bioavailability, and hepatoprotective potential of AG loaded in vesicles when compared with the unformulated drug. The effects of AG-loaded vesicles were comparable to those of silymarin, which is used as a standard drug [27]. Maiti et al. [28] obtained similar results in a study using the same formulation in rats. The formulation had an improved bioavailability and a better hepatoprotective activity than unformulated AG. The formulation was very helpful in solving the problem of rapid clearance and low elimination caused by the short half-life of AG. In an additional study, Sinha et al. [29] reported the capacity of AG encapsulated in liposome and decorated with mannosyl or fucosyl (as active targeting for macrophages) to reduce hepatic and renal toxicity. Furthermore, a decrease was observed in the parasitic burden of experimental leishmaniosis in a hamster model and in the splenic tissue histological architecture when compared with treatment with unformulated AG or conventional AG-loaded liposomes. In a further study, Li et al. [30] developed AG-loaded liposomal dry powder inhalers (LDPIs) for pulmonary delivery for the treatment of pneumonia induced by *Staphylococcus* (*S.*) *aureus*. The AG-loaded liposomes were freeze-dried to formulate LDPIs, and were found to be suitable for pulmonary delivery. A stronger *in vivo* anti-*S. aureus* pneumonic effect of the formulation was found at a tenfold dose, in comparison with unformulated AG or penicillin. The LDPIs significantly reduced the tumor necrosis factor α (TNF-α) and interleukin (IL)-1 pro-inflammatory cytokines. Phosphorylation of IκB-α in the nuclear factor-κB pathway was also inhibited to an extraordinary degree.

Piazzini et al. [31] developed liposomes for the central nervous system (CNS) delivery of AG by adding Tween 80 alone or in combination with didecyldimethylammonium bromide in order to modify the surface of the vesicles. The ability of liposomes to increase the permeability of AG was evaluated by a parallel artificial membrane permeability assay (PAMPA) and hCMEC/D3 cells. The size of the liposomes ranged from (96.4 ± 9.5) to (82.1 ± 9.3) nm, and the EE ranged from 44.7% ± 3.2% to 47.5% ± 3.3%. The liposomes showed excellent stability as suspensions or freeze-dried products. PAMPA and hCMEC/D3 transport studies revealed that all the developed liposomes increased the permeability of AG, in comparison with free AG. No alterations in cell viability were found. The main uptake mechanism was caveolae-mediated endocytosis, and the increased cellular internalization of the formulations was related to the positive charge [31].

2.2.4. Solid lipid nanoparticles

SLNs (Fig. 3) are formulated with physiological lipids, which are dispersed in water or in an aqueous surfactant solution. SLNs are typically spherical, with an average diameter between 10 and 1000 nm. The solid lipid core is stabilized by surfactants (emulsifiers) and formulated using fatty acids, monoglycerides, diglycerides, triglycerides, steroids, and waxes. All classes of emulsifiers can be used to stabilize the lipid dispersion, thus preventing particle agglomeration more efficiently [15].

AG encapsulated into SLNs was found to enhance antitumor activity in Balb/c mice because of an enhancement of bioavailability due to the improvement of the $AUC_{0-\infty}$ and C_{max} of AG in comparison with unformulated AG. A sustained-release pattern of AG-loaded SLNs was indicated by an increased value of T_{max} [32].

AG-loaded SLNs with an average diameter of 286.1 nm and a zeta potential of −20.8 mV were developed to improve AG bioavailability. The AG EE was 91.00%, while the drug loading was 3.49%. Both the bioavailability and anti-hyperlipidemic efficacy of AG-loaded SLNs were improved by increasing the stability and solubility of AG in the gastro-enteric tract. The bioavailability of AG loaded in SLN was increased to 241% in comparison with unformulated AG [33].

AG-loaded SLNs were formulated using Compritol 888 ATO and Brij 78. The EE was 92%. The SLNs showed exceptional physical and chemical stability at 4 and 25 °C after storage for one month. The SLNs were also stable when dispersed in human serum albumin and plasma. The *in vitro* release of AG-loaded SLNs at physiological pH was prolonged and sustained. The SLNs' ability to cross the BBB was evaluated *in vitro* by the PAMPA test and hCMEC/D3 cells in order to predict the passive transcellular permeability. The SLNs improved the permeability of AG in comparison with free AG, and the data from the two tests were comparable. Intravenously administered fluorescent SLNs were detected in brain parenchyma outside of the vessel, thus establishing their ability to cross the BBB [34].

2.2.5. Nanoemulsions and microemulsions

Both NEs and MEs (Fig. 3) are nanoscale emulsions characterized by high stability and transparency. However, the terms "ME" and "NE" are not interchangeable; an ME is an isotropic liquid mixture that forms spontaneously and is thermodynamically stable, whereas an NE is a nanoscale dispersion that is obtained by means of mechanical force and is only kinetically stable. The two phases of NEs and MEs may be water-continuous or oil-continuous. In addition, MEs can present bicontinuous systems (also called sponge-like systems) [15,35]. An AG-loaded NE was formulated in order to improve oral bioavailability and protective action against inflammatory bowel disease. The AG-loaded NE was prepared with water, ethanol, α-tocopherol, and Cremophor EL. The optimized AG-loaded NE was stable at 4 and 25 °C for three months, and had a droplet size of (122 ± 11) nm and a viscosity of 28 centipoise. An *ex vivo* test using an everted rat gut sac indicated that the jejunum was the optimal site for AG loaded in the NE. AG activity was 8.21 and 1.40 times higher than the values obtained with AG suspension and AG ethanol solution, respectively. The pharmacokinetic results showed a relative bioavailability of 594.3% when the AG-loaded NE was compared with an AG suspension. In addition, the ulcer index and histological damage score of indomethacin-induced intestinal lesions in mice were significantly reduced by pretreatment with AG-loaded NE [36].

An oil-in-water (O/W) ME loaded with AG was developed using isopropyl myristate, Tween 80, ethanol, and water. The mean droplet size was 15.9 nm, and the solubility of AG was 8.02 $mg \cdot mL^{-1}$. The formulation was stable, with a higher anti-inflammatory effect and bioavailability than AG tablets; it displayed no acute oral toxicity [37].

A special NE loaded with AG was prepared using layer-by-layer technology via electrostatic deposition of chitosan over alginate-encrusted O/W NE by means of ultra-sonication. The best stability was obtained after 20 min of sonication. The particle size of the multi-layered NE was measured to be within the range of 90.8–167.8 nm, with a zeta potential between 22.90 and 31.01 mV. The NE showed a strategic release pattern when assessed *in vitro* in various simulated biological fluids. It showed significant modulation in a liver function test (alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), and liver glycogen) and serum cytokines (IL-β, TNF-α, IL-10, and IL-6) when assessed *in vivo* in galactosamine-lipopolysaccharide-intoxicated mice, thus exhibiting significantly improved hepatoprotection [38].

2.2.6. Gold nanoparticles

Functionalized gold NPs (Fig. 3) are smart vectors for biomedical applications that can control geometrical and optical properties. Their large surface can be coated with diverse molecules (i.e., targeting agents, drugs, and anti-fouling polymers), making them versatile carriers. In particular, the targeted delivery of drugs is one of the most encouraging medicinal uses of gold NPs [39].

Engineered gold NPs loaded with AG, with a spherical geometry of 14 nm and a polydispersion index (PDI) value of 0.137, were developed. They exhibited resilient anti-leishmanial activity, against both wild-type (IC_{50} of (19 ± 1.7) $\mu mol \cdot L^{-1}$) and sodium stibogluconate (IC_{50} of (55 ± 7.3) $\mu mol \cdot L^{-1}$)/paromomycin (IC_{50} of (41 ± 6.0) $\mu mol \cdot L^{-1}$)-resistant strains. Complete macrophage uptake of AG gold NPs occurred within 2 h of exposure, and the cytotoxicity was significantly lower than that of amphotericin-B [40].

2.2.7. Nanocrystals and nanosuspensions

Ma et al. [41] designed a fast-dissolving nanocrystal-based solid dispersion to enhance the dissolution of AG. The nanodispersion was formulated using various ratios of hydroxypropyl methylcellulose (HPMC) and three super disintegrant excipients: sodium carboxymethyl starch, mannitol, and lactose. The dispersion with the highest concentration of HPMC exhibited the most rapid AG dissolution properties, probably due to the enhanced wettability. The performance of the super-disintegrants at a level of 20%, in combination with 25% HPMC, was tested. The formulation developed with 15% sodium carboxymethyl starch, 15% HPMC, and 10% lactose improved the dissolution (drug loading was up to 67.83% ± 1.26%). The *in vivo* pharmacokinetics studies revealed a significantly enhanced bioavailability of AG in comparison with unformulated AG. A nanodispersion was prepared and its performance tested. The dissolution rate (85.87%), C_{max} ((299.32 ± 78.54) $ng \cdot mL^{-1}$), and $AUC_{0-\infty}$ ((4440.55 ± 764.13) $mg \cdot (h \cdot L)^{-1}$) of the AG nanodispersion were significantly higher ($P < 0.05$) in comparison with crude AG. The $AUC_{0-\infty}$ was three times higher [42]. AG nanosuspensions containing glycyrrhizin had a mean particle size of 487 nm and exhibited excellent performance in comparison with those obtained with trehalose. The dissolution percentage of these AG nanosuspensions (99.87%) was significantly increased in comparison with that of unformulated AG (42.35%) [43].

In another study, an AG nanosuspension was prepared using *D*-α-tocopheryl polyethylene glycol 1000 succinate (TPGS, a surfactant that inhibits P-glycoprotein function) and sodium lauryl sulfate. The mean particle size was (244.6 ± 3.0) nm and the redispersibility index was 113% ± 1.14% ($n = 3$). Increased dissolution behavior was found; in addition, a Caco-2 cell monolayer test revealed that the membrane permeability (P_{app}) of AG in the nanosuspension was significantly higher than those of unformulated AG or AG nanosuspensions without TPGS ($P < 0.01$). Furthermore, the nanosuspension containing TPGS exhibited significantly higher C_{max} and $AUC_{0-\infty}$ ($P < 0.01$). A study using carrageenan-induced paw edema demonstrated that an AG nanosuspension containing TPGS was more effective and produced a greater increase in the serum levels of nitric oxide (NO), IL-1, and TNF-α ($P < 0.01$) and in superoxide dismutase (SOD) activity ($P < 0.05$) in comparison with unformulated AG [44].

Nanosuspensions were formulated with 3% AG, 5% poloxamer 188, 0.1% sodium tauroursodeoxy cholate, or 0.05% sodium deoxycholate, with 0.4 mm zirconium oxide pearls. These nanosuspensions showed a hexagonal morphology and a particle size of 300 nm, but no change in the crystalline habitus. A significant increase in saturation solubility was found, resulting in complete release within 0.25 h. The lyophilized formulation using mannitol (5%) as a cryoprotectant had respectable physical and chemical stability during the six-month storage period. Pharmacokinetic and tissue distribution studies revealed that the AG was mainly distributed in the liver and was rapidly eliminated from the blood [45].

3. Conclusions

Microcarriers and nanocarriers are a tremendously important field of research in bioactive constituents, which include natural products and herbal extracts. The use of these technologies has already had a significant impact on many areas of medicine by allowing appropriate therapeutic treatments of certain essential drugs (principally antitumor and antiparasitic drugs). In addition to its numerous potential activities, AG, which is the characteristic constituent of *Andrographis paniculata* (Burm.f.) Nees, has a low bioavailability, limited biodistribution and localization, lack of stability in gastrointestinal environments, and very short biological half-life. Remarkable results have been obtained in improving AG bioavailability, target-tissue distribution, and efficacy using micro- and nano-formulations based on AG; these achievements

represent a decisive step toward establishing a key role for AG in modern clinical treatment.

In comparing the formulations reported in this review, it can generally be stated that nanocarriers have certain advantages over micro-delivery systems: They are more stable, provide more surface area, and are able to overcome anatomical barriers and physiological clearance mechanisms. Therefore, with nanocarriers, AG activity becomes enhanced, more detectable, and prolonged. Each nanoparticle has its own advantages, disadvantages, and characteristics.

The use of nanopowders is the simplest and most affordable technique to enhance AG solubility. This approach is typically carrier-free, and is the most suitable technology for compounds with $P_{app} < 10^{-5}$, a high melting point, and high doses, as in the case for many natural products. The advantages of this approach include improved absorption after oral, mucosal, and parenteral administration; the ability to control the rate and extent of drug absorption; low cost; and easy scale-up. However, the limitations of this approach are numerous, because these nano-formulations are not able to enhance photostability or chemical stability, overcome multidrug-resistance phenomena, or impart passive and active targeting.

Both lipid and polymeric NPs can overcome all the limitations of nanopowders; in particular, polymeric NPs can address biocompatibility and low biodegradability and their resulting safety issues, especially after long-term administration. A main limitation of SLNs is their low loading capacity and leakage during storage. This limitation can be overcome by using the new improved generation of lipid nanocarriers—the so-called nanostructured lipid carriers—which have a higher loading capacity and somewhat less water in the dispersion due to more complex lipid mixtures and the presence of both solid (fat) and liquid (oil) lipids at ambient temperature within the hydrophobic core. The use of these nanovectors for AG has not been reported in the literature; however, they could be a suitable tool to further improve the therapeutic use of AG.

Vesicles are very versatile nanocarriers and offer many advantages: Both hydrophilic and hydrophobic drugs can easily be encapsulated in vesicles due to the presence of the hydrophilic compartment and lipophilic palisade, thus increasing their bioavailability, delaying their metabolism, and prolonging their circulation lifetime. In addition, both passive and active targeting can be achieved using vesicles. The disadvantages and limitations of vesicles include their leaky nature, which leads to premature drug release; their poor encapsulation efficiency; the fact that they are quite expensive; and their low utility after oral administration.

Both MEs and NEs are interesting nanovectors because they are able to load high doses of a drug (up to 20%, w/w) and present many advantages over other nanosystems. They can be administered by all routes, control the rate and extent of drug absorption, increase solubility and bioavailability, enhance photostability and chemical stability, and overcome multidrug-resistance phenomena. In addition, MEs and NEs are superior over nanocarriers due to their low cost and easy scale-up.

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Compliance with ethics guidelines

Marta Casamonti, Laura Risaliti, Giulia Vanti, Veri Piazzini, Maria Camilla Bergonzi, and Anna Rita Bilia declare that they have no conflict of interest or financial conflicts to disclose.

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