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Review Article

Potentials of human bile acids and their salts in pharmaceutical nano delivery and formulations adjuvants

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Abstract. In the last decade, the attention of the scientific community has been focused on bile acids and their salts as systems for the transportation of drugs; specifically their role as carriers and integration into nanomedicine. Bile acids can play a critical role as drug carriers in the form of chemical conjugates, complexation, mixed micelles formation as well as stabilized bile acid liposomes (bilosomes). The unique molecular structure and interaction of these amphiphilic-steroidal compounds make them an interesting subject of research. This review is based on literature research in order to emphasize the importance of bile acids and their salts as absorption modulators in order to improve therapeutic potentials of low bioavailability drugs.

Keywords: Bile acids, bilosomes, nano-structures, absorption enhancers, drug delivery

1. Introduction

Nanotechnology is truly a multidisciplinary science where chemists, physicist, biologists and pharmaceutical scientist all have played major roles to develop novel treatment and diagnosing modalities. It is evident through this review that application of nanotechnology in drug delivery and medicine has

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paved new pathways and opened many doors for providing customizable and safer treatment option. Ultimately, through the manipulation of molecular size and surface properties, researchers are able to deliver drugs for longer period of time with less frequent dosing (sustained release) and with greater precision and penetration in difficult to access tissues. The development of nanoparticle-based drug formulations has yielded the opportunities to address and treat challenging diseases [1].

2. Bile acids and their salts as absorption modulators

The structure of bile acids allows for the addition of various drugs to one of their functional groups, including hydroxyl groups. In accordance with predictions of quantitative relationships between the structure and activity (SAR studies), the most effective molecular recognition by membrane hepatocyte transporters and ileal enterocytes is achieved by linking the drug to the C3 position [2–4].

Such conjugates demonstrate interactions with bile acid transporters, confirming the hypothesis that bile acids in the form of a "Trojan horse" may alter the pharmacokinetic and pharmacodynamic properties of the drug they carry. Research into the biology of transporters was strongly stimulated by available technologies for the study of the function and structure of transport proteins, which revealed their importance for the detection and development of drugs, especially for their absorption and disposition. Physiological transport systems are studied as potential carriers in order to improve absorption and permeation of the membrane and achieve the effect of organ-specific drugs. In particular, bile acid transport systems in the liver and small intestine and oligopeptide carriers are of great importance for the molecular delivery of drugs [5].

Recently, a unique class of amphiphilic copolymers has been introduced into the field of cancer targeting and gene distribution by bile salts. Such polymers are composed of a linear hydrophilic biodegradable branch (such as PEG [polyethene glycol], dextran, pullulan, chitosan and PLGA (polylactic-glycolic acid)) and a flexible linear double-stranded holic acid oligomer as a nucleus-forming hydrophobic block. Owing to the surface hydrophilicity, such amphiphilic structures would spontaneously form self-assembling nanoaggregates in water induced by an intramolecular and/or intermolecular bond between the hydrophobic segments in order to reduce free energy on the contact surface. Accordingly, biodegradable bile acid nanoparticles have a positive potential in drug distribution [3–6].

Nanoparticles based on bile salt polymer conjugates can be successfully filled with lipophilic chemotherapy drug paclitaxel (PTX) with a high drug concentration, high antitumor activity and high stability compared with market products (Taxol® and Abraxane®) [3]. In research conducted by Li and coauthors was developed [7]. This system is based on the micelles formed by a new class of well-defined linear PEGylated chain of two cholic acid oligomers in aqueous solution. By varying the length of the linear PEG chains and holic acid oligomer configuration, the physical and chemical properties of the amphiphilic polymer and the resulting micelles can be easily fine-tuned. In addition, the study states that nanocarriers with PTX filling are much more stable than Abraxane (PTX/human serum albumin nanoaggregate). The PTX release profile from the micelles showed that the anti-tumour activity of the nanoparticles applied onto PTX against the ovarian cell line *in vitro*, with continuous exposure to the drug, is similar to Taxol (a formulation of PTX dissolved in Cremophor EL and ethanol) or Abraxane. Targeted delivery of the drug to the tumour site with these new micelles was demonstrated by Near-Infrared Fluorescence (NIRF) in bare mice with ovarian cancer xenograft. Moreover, nanocarriers with PTX showed superior anti-tumour efficacy compared to Taxol in the equivalent dose of PTX.

Using a lipophilic cancer drug (epirubicin) (EPB) inserted into the hydrophobic part of deoxycholic acid conjugate – hydrophobically modified carboxymethylated-curdlan (DCMC) – was developed as a

new anticancer drugs carrier [8]. *In vitro* release of EPB from DCMC-self-settled nanoparticles showed continued drug release. Compared to free drug, EPB-filled DCMC nanoparticles showed greater cytotoxicity, which can be attributed to increased cellular intake. *In vivo* toxicity study has shown that the DCMC conjugate did not cause unexpected side effects. The study has shown that, compared to free drug, DCMC increases EPB intake into the tumour and reduces EPB intake into the kidneys and heart.

Despite benefit of polymeric bile salt nanoaggregates as parenteral carcinoma treatment, this is not the case with oral administration. Although the oral route is most appropriate, the poor oral bioavailability of drugs is a common handicap, which is usually resolved through phospholipid-based nanoparticles [3].

3. Mixed micelles as drug carriers

To improve absorption, drugs with poor water solubility can be dissolved within bile micelles. Micelles as drug carriers have certain advantages such as the ability to solubilize drugs and thereby increase their bioavailability, can be retained in the body (in the bloodstream) for a time sufficient to allow gradual drug accumulation within the intended organ. Their size allows them to accumulate in parts of the body with a permeable circulatory system and they can serve to target a specific place by fixing a specific ligand to the outer surface and can be easily and reproducibly prepared in large quantities. In micellar form, the drug (primarily a poorly soluble drug) is well protected against possible inactivation due to biological environment, not causing side effects.

It is known that non-ionic surfactants (PAM) micelles have anisotropic water distribution within their structure – the water concentration decreases from the surface towards the micellar core. Due to anisotropy, micelles show a polar gradient from a highly hydrated surface to a hydrophobic core. As a consequence, the spatial position of a particular solubilized substance within the micelle depends on its polarity. In aqueous systems, non-polar molecules will be placed in the micelle core, polar molecules will be absorbed onto the surface of the micelle, and the medium polarity substances will be distributed along with the PAM molecules. The capacity of the implantation of a particular PAM depends on various factors such as the chemical structure of the drug and PAM, drug polarity, the position of the drug within the micelle, temperature, pH, etc. The capacity of the implantation of a particular PAM depends on various factors such as the chemical structure of the drug and PAM, drug polarity, the position of the drug within the micelle, temperature, pH, etc. At the same time, the increase in the micelle core decreases Laplace pressure, which is a result of the curvature of the intermediate layer, and also facilitates the incorporation of the hydrophobic substance into the micellar core [9].

Mixed micellar systems composed of bile salts, phospholipids, fatty acids and polyamines can have improved properties in terms of transport promotion, allowing at the same time the reduction of bile acid concentration and, consequently, potential membranolytic effects.

In 2011, Maestrels and co-workers tested the influence of chitosan (CS), bile acids and their NaDHC sodium salts on NSAID oxaprozin, which has very poor water solubility [10]. Addition of bile components improved the drug dissolution rate. The presence of CS enabled a significant increase in permeability of the drug through the Caco-2 cells in relation to drugs without added components. The combined use of CS and NaDHC could be used to develop effective oral dosage forms of oxaprozin, with increased solubility and permeability of the drug, and subsequently improved bioavailability. Mahajan and Mahajan examined the effect of phenothiazine drugs with bile salts using sodium cholate and sodium deoxycholate and confirmed the increased bioavailability of drugs [11]. Mixed micelles show advantages in parenteral administration, given their small size (usually below 60 nm). In addition, they are not suitable for the incorporation of hydrophilic drugs, as opposed to the phospholipid vesicles. These

deficiencies may explain a limited number of mixed micelle-based drugs that have been placed on the market of pharmaceutical products so far [12].

4. Liposomes, niosomes and bilosomes

Liposomes are drug transport systems in which the drug is encapsulated within closed, spherical vesicles composed of one or more concentric lipid layers around the central hydrophilic core. The specific structure of liposomes enables the incorporation of liposoluble drugs into the lipid layer as well as the hydrosoluble substances into the inner hydrophilic phase, as well as peptides and small proteins into the lipid-aqueous system. Orally distributed liposomal carriers can improve the drug solubility and protect encapsulated therapeutic agents from extreme conditions in the gastrointestinal tract. Liposomes, with their membrane liquid lipid bilayer and their application size, can significantly improve oral absorption. Unfortunately, the clinical use of conventional liposomes is hampered by their poor stability in the gastrointestinal tract. Although liposomes stabilize proteins within the gastrointestinal tract after oral application to a certain degree, the effect of acidity of gastric juice, pancreatic enzymes and bile salts show negative effects [13].

Self-organization of non-ionic amphiphilic molecules in water forms closed dual layer structures or niosomes structures, in which the hydrophobic parts of the molecule are sheltered and the hydrophilic groups are in maximum contact with water. By their structure, niosomes are analogous to phospholipid vesicles (liposomes) being able to incorporate molecule drugs and serve as carriers [14].

Niozomes contain a surfactant, which improves the stability of the drug delivery system. This non-ionic surfactant belongs to the class of alkyl or dialkyl polyglycerol ether and cholesterol with subsequent hydration in an aqueous medium. They can improve the therapeutic effect of peptides by reducing the minimum clearance time from the systemic circulation, by increased bioavailability and targeted and controlled delivery of the drug to the site of action.

Polymersomes are nanoparticles that demonstrate a similar general structure and function, such as liposomes and niosomes. They can be used to absorb both hydrophilic and hydrophobic compounds. However, unlike liposomes and niosomes, instead of phospholipids or any amphiphilic surfactant for forming the shell, the amphiphilic block copolymers were used, i.e. polymers consisting of covalently bound hydrophilic and hydrophobic chains. An example block copolymer are three-block copolymers poly(caprolactone) -poly(ethylene glycol)-poly (caprolactone). The construction of the polymeric vesicle, unlike the lipid-based vesicle, provides several advantages including controlled release of the compounds and increased shell stability. In addition, a molecular-dynamic study has shown that the amphiphilic block copolymer also has the ability to form cell membranes and vesicles. Polymersomes can be synthesized by methods similar to those used for polymer nanoparticles [15].

New vesicular dosing systems containing bile salts and liposomes are known as bilosomes, which act as penetration enhancers. The incorporation of bile salts into liposomes can stabilize the membrane against the harmful effects of physiological bile acids in the gastrointestinal tract and facilitate the internalisation of particles. In addition, bilosomes are produced from natural lipids making them biocompatible [16].

5. Oral vaccines and hydrophilic active substances

Oral vaccines provide significant benefits as non-invasive drug-free treatment, which is easier for the patient and less susceptible to cross-contamination. However, the pharmaceutical administration of this

route of administration is impeded by a gastrointestinal environment that is harmful to many vaccine formulations. Researchers have been recently dealing with bilosomes since new colloidal agents, such as Cholera toxin vaccine, toxoid diphtheria vaccine, recombinant baculovirus vaccine, influenza vaccine, hepatitis B vaccine and others have attracted attention in the field of oral immunization [3].

Bile salts are identified as topical penetration enhancers owing to the membrane destabilization. The research conducted by Conacher and co-authors in 2001 into the ability of non-ionic surfactant vesicles to induce a systemic immune response in mice after oral immunization was conducted using a standard antigen (bovine serum albumin), synthetic measles peptide and influenza vaccine [17]. It is observed that the efficacy of this formulation has been significantly increased by including bile salts (especially deoxycholate) in the formulation, i.e. formation of bilosomes. It is particularly important that these studies show that oral administration of bilosomes incorporating a vaccine can induce antibodies as a potent response just like a parenterally administered vaccine containing the same amount of antigen. As biosomes are prepared from natural lipids and do not have obvious toxicity associated with their use, they represent a useful modification of conventional systems based on lipid vesicles for the oral delivery of proteins and peptides. The positive effect of bilosomes after oral administration of vaccines was also confirmed in a study which included the conjugation of a bilosomal system to the cholera subunit of toxin B (CTB) [18]. In 2010, a study was done to examine the stability and potential of glucomannan-modified bilosomes (GM-bilosomes) in the induction of an immune response after oral administration. GM-bilosomes maintained the chemical and conformational stability of tetanus toxoid (TT). It was shown that GM-bilosomes did not only provoke significantly higher systemic immune responses compared to bilosomes, niosomes and orally administered, adsorbed TTs, but they also showed the induction of immune mucous response, as well as immune cell-mediated responses that had not been induced by conventional immunization [19]. Similar studies on Human Enterovirus 71 (HEV71) and cholera toxin B21 have shown that bilosomes have inherent adjuvant properties when linked to antigen and that bilosomes can be a good candidate for an oral vaccine against these infections [20,21].

Given the above, it can be said that bilosomes and modified bilosomes (including probiosis and surface-structured bilosomes) show greater oral stability, protective effect and characteristics that improve permeation compared with conventional liposomes and niosomes. Such new nanocarriers can be used to improve the oral bioavailability of vaccines, hydrophilic drugs and insoluble active substances.

Oral vaccination with bilosomes was proposed as a non-invasive, painless approach to immunization, which consequently improved conscious adherence to therapy and led to increased vaccination coverage. In addition, it is said that bilosomes provide a safer surrogate for diluted oral vaccines with less chance of regression to virulence [3].

6. Orally administered hydrophobic drugs

The suitability of bilosomes to improve oral availability was not limited to hydrophilic drugs within the hydrophilic core. Several hydrophobic active substances were successful candidates for the formulation in the form of bilosomes as well as in cases when the drug would be within phospholipid vesicle bilayer [3].

In this context, several autors published the results of studies performed with fenofibrate and cyclosporin A, as extremely hydrophobic substances [22,23]. The main purpose of these studies was to evaluate the oral bioavailability of these substances when liposomes containing bile salt were used as oral drug release systems. *In vitro* experiments showed an extremely low degree of active substances

release from formed bilosomes in relation to the release from micronized fenofibrate capsules i.e. conventional cyclosporin A liposomes. Contrary to *in vitro* results, *in vivo* measurements of pharmacokinetics and bioavailability showed higher rates of bilosome absorption in both studies [3]. The disparity between oral bioavailability and *in vitro* release with liposomes suggests that bile salts can be used to increase the oral bioavailability of poor water-soluble drugs.

A weak *in vitro/in vivo* correlation of oral liposomal formulations implied a potential deficiency *in vitro* release as a means of assessing bilosomes. *In vitro* release media are usually deprived of any biosurfactants, enzymes, or bile contents and, therefore, they implausibly simulate *in vivo* environments. The *in vitro* analysis did not take into account possible disorder of the liposomal vesicular structure in physiological conditions [3].

An improvement in glycemia was demonstrated in the study on the bilosom as a system for insulin delivery. Ayogu and his team formulated and tested two bilosomal preparations. One was prepared using lipid extract from soybean seed (SBE), palmitic acid (PA) and cholesterol (CH), while the other (BII) contained PA and CH as a lipid component [24]. Each of the preparations contains 0.5% sodium deoxycholate (NaDC) and 0.5% soluble insulin. BI was administered only orally, while BII was administered subcutaneously, intraperitoneally and orally to different groups of male rats treated with streptozotocin, while blood glucose levels were measured at predetermined time intervals. The results of the studies showed that the oral administration of BI and BII caused a decrease in blood glucose, which could mimic the endogenous insulin release with prolonged activity, but also a lower reduction in blood sugar compared to the parenteral administration. BI (oral I) containing SBE had a greater blood glucose reduction than BII (oral II), indicating that SBE increased the bioavailability of insulin. The formulation of bilosomal insulin may provide a good system for oral administration of insulin that would affect the entero-insular axis similar to endogenous insulin.

Elnaggar Y.S. states that, given the complexity of gastrointestinal physiology, the exact mechanism of the increased bioavailability of oral bilosomes has not been fully clarified to date [3]. It can be said that this is a consequence of the interaction between many factors including the protective effect in relation to severe gastrointestinal conditions, the ability to fluidise the membrane, and the physical and chemical properties of the incorporated bile salt. In comparison with conventional vesicles, bilosomes had significantly higher stability and protective properties compared to gastric enzymes, pH and bile content. On the other hand, a hypothesis was proposed on the formation of a lipophilic ion pair between the bile salts and the hydrophilic active ingredient. Thereby, the increase in the passive diffusion of water-soluble molecules across biological membranes and the membrane fluidizing effect of bile salts would also justify the improved bioavailability. Such complexes of ionic pairs would also be beneficial in terms of increasing the efficiency of the effect of cationic hydrophilic drugs in the targeted place due to the formation of multiple lipophilic compounds and greater affinity for phosphatidylcholine [3].

7. Non-oral administration of bilosomes

Although bilosomes were not proposed for transdermal drug delivery, it seems that the carriers of this type have a promising potential in this respect. Transferosomes are ultradeformable lipid vesicles containing various effective activators, including bile salts. Transferosomes containing bile salts have shown efficacy in improving the transdermal permeability of cosmetic and pharmaceutical molecules when used in concentrations below 0.2% [25].

This is also confirmed by the research of Mahallawi and coworkers conducted to assess the ability and safety of the use of bilosomes for the transdermal delivery of tenoxicam (TX) [26]. Both, *ex vivo*

permeation and *in vivo* studies confirmed the superiority of bilosomes in relation to the drug solution in the transdermal delivery of TX. In addition, an *in vivo* histopathological study has demonstrated the safety of locally applied bilosomes. The results confirmed that bilosomes could be further adopted for transdermal drug delivery.

A great lack of research was observed to deal with the potential of bilosome distribution through local administration methods. For example, the use of bilosomes in corneal permeability is a new area that has not been fully investigated so far.

Although bilosomes have proven to be a successful carrier of cationic water-soluble active substances, loading sufficient amounts of the anionic active ingredient remains an obstacle. Taking into account the negative charge and hydrophilicity of the bile salt, the incorporation of the active cationic substance would keep the gallbladder in the bilayer to achieve the effect of membrane stabilization. Nonetheless, the incorporation of anionic hydrophilic drugs would be accompanied by low binding effectiveness, migration of both hydrophilic bile salts and active substances to the outer phase, stimulating the migration of surfactant into type IV of the lipid formulations system. Lipid formulations of type IV contain only surfactants in their composition and represent the so-called surfactant systems [3].

8. Oral drug administration with bile acids as carriers

The oral bioavailability of some drugs is very low due to enzymatic degradation in the gastrointestinal tract and poor permeation through the intestinal epithelial cells. One way to overcome this problem is to use absorption enhancers. Song, Chung, Shim investigated the improvement of the intestinal salmon calcitonin absorption (sCT) from proliposone containing bile salts [26]. It has been said before that it is known that some of the bile salts, including NaTDC, form lipophilic complexes of ionic pairs with different organic cations, which increases cation permeability in biological membranes. Since sCT is a hydrophilic and cationic molecule, it is shown that the effect of NaTDC in the increase in sCT permeability could be due to the ability to form ionic pairs of bile salts with sCT. Eskandar et al. [14] stated that the findings of Songa et al. [27] are in contrary to the findings of Cetina et al. [28] in which no significant difference was found between formulations.

9. Transdermal use of drugs with bile acids as carriers

One of the ways to increase the absorption of drugs through the skin is the use of bile salts as an absorption enhancer. Many studies have been performed in this respect, showing positive effects of bile salts on transdermal absorption. One study was conducted to assess the suitability of sodium-deoxycholate (Na-DOC) gels containing betamethasone-17-valerate (BMV) for topical administration. The study confirmed *in vitro* the flux BMV from Na-DOC gels on the rat skin was 2.5 (0.05% gel) and 8.5 times (0.1% gel) higher than the commercial cream (0.1%), respectively. Pharmacodynamic responses after *in vivo* administration in rats were also determined. A significant correlation was observed between anti-inflammatory activity and *in vitro* permeation of BMV. Na-DOC gels had significantly higher oedema inhibition in comparison to commercial cream at all-time intervals. Besides, according to histological studies, Na-DOC gel has no irritating effect on the skin. In conclusion, the Na-DOC gel formulation can be proposed as a promising alternative system for topical BMV administration [29].

In another set of experiments transdermal aminophylline absorption was analysed. *In vitro* experiments on percutaneous absorption were performed on snakeskin using Franz Cells. The flux (J),

permeability coefficient (P) and the gain factor (EF) for each formulation were calculated. Sodium-tauroglycocholate (NaTGC) (100, 200 and 500 μg/mL), lauric acid (1.7 and 15%) and ethanol (60%) were used as enhancers. The results showed that all enhancers – NaTGC to the greatest extent – significantly increased drug permeability and EF [30]. Very similar results were obtained by Moghimipour and coworkers who examined the effect of NaTGC and NaDC bile salts as the transdermal theophylline absorption enhancer through snakeskin, individually and in combination [31]. Percutaneous *in vitro* absorption experiments were performed on snakeskin using Franz diffusion cells. The surface activity of bile salts was also determined. Bile salts significantly increased transdermal absorption of theophylline through the treated skin model compared to the control group, although the effect of NaTGC was more significant. Similarly, a reduction in the enhanced effect was observed after mixed administration of bile salts. It was concluded that the increased effect of bile salts depends not only on nature and concentration but also the type and character of the gallbladder used.

10. Nasal use of drugs with bile acids as carriers

The nasal route of administration is suitable for small doses showing poor stability in the gastrointestinal tract. Despite many benefits of the nasal route, the restrictions such as high molecular weight (HMW) of drugs may interfere with drug absorption through the nasal mucosa. Recent studies have specifically focused on the nasal application of HMW therapeutic agents such as peptide and protein drugs and vaccines intended for systemic effects. Due to their hydrophilic structure, the nasal bioavailability of peptide and protein drugs is normally less than 1%. Apart from the poor mucosal permeability and enzymatic degradation in the nasal mucous membrane, these drugs are, due to muscular clearance, quickly removed from the nasal cavity after administration. There are many approaches to increase the retention time of drug formulations in the nasal cavity, which results in increased drug absorption. Different approaches, including the use of absorption enhancers, have been tried to overcome the problem that limits the nasal absorption of drugs [32,33].

Some researchers studied the role of bile salts absorption enhancers for nasal administration of insulin [14,34,35]. As early as in 1981 it was shown that bile salts could improve the transport of insulin through the nasal mucosa by slowing down the degradation of insulin using leucine aminopeptidase-proteolytic enzyme [34,35]. Uchida and associates examined the effect of bile salt on insulin permeability through the nasal cavity of rabbits. In addition to the confirmed increase in permeability, they found that sodium glycodeoxycholate (NaGDC) was more effective as a nasal absorption enhancer in the nasal mucosa than sodium glycocholate (NaGC), due to the lack of a hydroxyl group at seven steroid positions, which increased its lipophilicity. However, NaGC is widely used as a nasal absorption enhancer due to relatively low toxicity compared to other bile salts [36].

11. Bucal use of drugs with bile acids as carriers

During the last decade, there was a particular interest in the delivery of medicines in the buccal mucosa, the aim of which was to increase the bioavailability of different controlled drug delivery systems (CDDSs). Besides, drugs demonstrating poor bioavailability, when taken orally, are practical for buccal administration. However, the buccal mucosa is less permeable and therefore cannot ensure rapid absorption. Many compounds were tested as absorption enhancers, including bile salts, surfactants, fatty acids

and derivatives, ethanol, cyclodextrins and chitosans [37]. Hoogstraate and coauthors examined the *in-vitro* effect of enhancing the penetration of bile salts on the transport of hydrophilic macromolecular compounds via swine buccal mucosa [38]. Simultaneous administration of 100 mM trihydroxy sodium glycollate (NaGC) bile salts and sodium taurocholate (NaTC) and sodium glycodeoxycholate dihydroxy bile salts (NaGDC) and sodium taurodeoxycholate (NaTDC) increased the *in vitro* transport of fluorescein isothiocyanate (FITC) factor of one hundred or more without significant differences between the four bile salts. It can be concluded from these results that the diluted di- and trihydroxide bile salts increased the transport of hydrophilic compounds via buccal epithelium below 10 mM by increasing intercellular transport to 10 mM and above by opening the transcellular pathway. In 2007, Mahalingam and associates conducted a similar study by examining the effect of bile acids on the intake of 5-aza-2'-deoxycytidine (decitabine) via swine mucosa, which was evaluated as an alternative to the complex intravenous infusion system currently used to administer the drug. Trihydroxylic bile salts significantly increased decitabine flow at a concentration of 100 mM (P 9.05). Two dihydroxylic bile salts, sodium deoxytaurocholate and sodium deoxyglycolate, have shown a better effect than in trihydroxylic bile salts. It is noted that improvements in the flow of decitabine in the presence of bile salts may occur due to a complex process involving solubilization and micellar absorption of intercellular lipids, denaturation and protein extraction, inactivation of enzymes and tissue swelling [39].

12. Rectal use of drugs with bile acids as carriers

In study of Kima and collaborators, the potency of sodium taurocholate (NaTC) bile salts in improving the bioavailability and anti-tumour efficiency of docetaxel (DCT) in rectal administration was analysed. They designed nanomicelles as drug distribution systems composed of DCT/poloxamer 407 (P407)/poloxamer 188 (P188) Tween 80/NaTC to improve the bioavailability and antitumour efficacy of DCT after the rectal administration. Poloxamer was selected as the formulation of unique thermosensitive and bioadhesive nanomicelles charged with DCT. Pharmacokinetic results showed that NaTC had influenced the increase in half-life and plasma DCT levels. Although elevated levels of DCT in plasma from the group of nanomicelles with bile salts did not improve the antitumour potential, DCT-charged nanomicelles can reduce side effects associated with drugs such as hypersensitivity reactions and fluid retention, while retaining potential antitumour efficacy in clinical subjects [40].

13. Conclusion

This article highlighted the importance of bile salts as absorption modulators with an emphasis on nanostructures, as well as a review of studies on drug formulations with bile acids as carriers intended for the targeted treatment of tumour diseases, antimicrobial therapy and vaccination. Therapeutic nanosystems that will improve the pharmacokinetic properties of the drug, enable controlled release and targeted delivery and reduce adverse effects and toxicity profiles are of great medical significance.

Bile acids and their salts, due to their ability to facilitate the transportation of drugs through biological membranes, are very important for drug absorption and can be considered as auxiliary agents in the drug formulation. By incorporating into bilosomes, mixed micelles and chemical conjugates, bile acids alter the pharmacokinetic properties of the drug and improve its bioavailability thus enabling a suitable targeted therapy of numerous diseases.

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Conflict of interest

None to report.

References

[1] Rizvia SAA, Salehb AM. Applications of nanoparticle systems in drug delivery technology. Saudi Pharm J. 2018 Jan; 26(1): 64-70.
[2] Stojančević M, Pavlović N, Goločorbin-Kon S, Mikov M. Application of bile acids in drug formulation and delivery. Front Life Sci. 2013; 7(3-4): 112-22.
[3] Elnaggar YS. Multifaceted applications of bile salts in pharmacy: An emphasis on nanomedicine. Int. J. Nanomed. 2015; 10: 3955-71.
[4] Baringhaus KH, Matter H, Stengelin S, Kramer W. Substrate specificity of the ileal and the hepatic Na(+)/bile acid cotransporters of the rabbit. II. A reliable 3D QSAR pharmacophore model for the ileal Na(+)/bile acid cotransporter. J Lipid Res. 1999; 40(12): 2158-68.
[5] Kramer W. Transporters, trojan horses and therapeutics: suitability of bile acid and peptide transporters for drug delivery. Biol Chem. 2011; 392(1-2): 77-94.
[6] Gautrot JE, Zhu XX. Biodegradable polymers based on bile acids and potential biomedical applications. J Biomater Sci. 2006; 17(10): 1123-39.
[7] Li Y, Xiao K, Luo J, Lee J, Pan S, Lam KS. A novel size-tunable nanocarrier system for targeted anticancer drug delivery. J Control Release. 2010; 144(3): 314-23.
[8] Gao F, Li L, Zhang H, Yang W, Chen H, Zhou L, et al. Deoxycholic acid modified-carboxymethyl curdlan conjugate as a novel carrier of epirubicin: *in vitro* and *in vivo* studies. Int J Pharm. 2010; 392(1-2): 254-60.
[9] Torchilin VP. Structure and design of polymeric surfactant-based drug delivery systems. J Contr Rel. 2001; 73: 137-72.
[10] Maestrelli F, Cirri M, Mennini N, Zerrouk N, Mura P. Improvement of oxaprozin solubility and permeability by the combined use of cyclodextrin, chitosan, and bile components. Eur J Pharm Biopharm. 2011; 78(3): 385-93.
[11] Mahajan S, Mahajan RK. Interactions of phenothiazine drugs with bile salts: micellization and binding studies. J Colloid Interface Sci. 2012; 387(1): 194-204.
[12] Rupp C, Steckel H, Muller BW. Mixed micelle formation with phos¬phatidylcholines: the influence of surfactants with different molecule structure. Int J Pharm. 2010; 387(1-2): 120-8.
[13] Nguyen TX, Huang L, Gauthier M, Yang G, Wang Q. Recent advances in liposome surface modification for oral drug delivery. Nanomedicine (Lond). 2016; 11(9): 1169-85.
[14] Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm. 1998; 172: 33-70.
[15] Ishak KA, Mohamad Annuar MS, Ahmad N. Nanotechnology Applications in Food: Chapter 9: Nano-delivery Systems for Nutraceutical Application, Academic Press, 2017 str. 179-202.
[16] Eskandar M, Abdulghani A, Somayeh H. Absorption-Enhancing Effects of Bile Salts. Molecules. 2015; 20: 14451-73.
[17] Conacher M, Alexander J, Brewer JM. Oral immunisation with peptide and protein antigens by formulation in lipid vesicles incorporating bile salts (bilosomes). Vaccine. 2001; 19(20-22): 2965-74.
[18] Shukla A, Katare OP, Singh B, Vyas SP. M-cell targeted delivery of recombinant hepatitis B surface antigen using cholera toxin B subunit conjugated bilosomes. Int J Pharm. 2010; 385(1-2): 47-52.
[19] Premanand B, Prabakaran M, Kiener TK, Kwang J. Recombinant baculovirus associated with bilosomes as an oral vaccine candidate against HEV71 infection in mice. PLoS One. 2013; 8(2): e55536.
[20] Singh P, Prabakaran D, Jain S, Mishra V, Jaganathan KS, Vyas SP. Cholera toxin B subunit conjugated bile salt stabilized vesicles (bilo¬somes) for oral immunization. Int J Pharm. 2004; 278(2): 379-90.
[21] Jain S, Harde H, Indulkar A, Agrawal AK. Improved stability and immunological potential of tetanus toxoid containing surface engi¬neered bilosomes following oral administration. Nanomedicine. 2014; 10(2): 431-40.
[22] Chen Y, Lu Y, Chen J, Lai J, Sun J, Hu F, et al. Enhanced bioavailability of the poorly water-soluble drug fenofibrate by using liposomes containing a bile salt. Int J Pharm. 2009; 376(1-2): 153-60.

[23] Guan P, Lu Y, Qi J, Niu M, Lian R, Hu F, et al. Enhanced oral bioavailability of cyclosporine A by liposomes containing a bile salt. Int J Nanomedicine. 2011; 6: 965-74.
[24] Ayogu IJ, Ogbonna O, Ayolugbe CI, Attama AA. Evaluation of the pharmacodynamic activity of insulin from bilosomal formulation. Curr Drug Deliv. 2009; 6(4): 415-8.
[25] Paolino D, Cosco D, Cilurzo F, Trapasso E, Morittu VM, Celia C, et al. Improved *in vitro* and *in vivo* collagen biosynthesis by asiaticoside-loaded ultradeformable vesicles. J Control Release. 2012; 162(1): 143-51.
[26] Al-Mahallawi AM, Abdelbary AA, Aburahma MH. Investigating the potential of employing bilosomes as a novel vesicular carrier for transdermal delivery of tenoxicam. Int J Pharm. 2015; 485(1-2): 329-40.
[27] Song, KH.. Chung SJ, Shim CK. Enhanced intestinal absorption of salmon calcitonin (sCT) from proliposomes containing bile salts. J Control Release. 2005; 106: 298-308.
[28] Cetin M, Aktas MS, Vural I, Ozturk M. Salmon calcitonin-loaded Eudragit® and Eudragit®-PLGAnanoparticles: *In vitro* and *in vivo* evaluation. J. Microencapsul. 2012; 29: 156-66.
[29] Senyigit T, Tekmen I, Sönmez Ü, Santi P, Özer Ö. Deoxycholate hydrogels of betamethasone-17-valerate intended for topical use: *In vitro* and *in vivo* evaluation. Int J Pharm. 2011; 403: 123-29.
[30] Kouchak M, Handali S. Effects of various penetration enhancers on penetration of aminophylline through shed snake skin. Jundishapur J Nat Pharm Prod. 2014; 9: 24-9.
[31] Moghimipour E, SajjadiTabassi SA, Kouchak M, Varghaei H. Combination strategies for enhancing transdermal absorption of theophylline through shed snake skin. Asian J Pharm Clin Res. 2012; 5: 30-34. Molecules 2015, 20 14470.
[32] Merkus FWHM, Schipper NGM, Hermens WAJJ, Romeijn SG, Verhoef JC. Absorption enhancers in nasal drug delivery: Efficacy and safety. J Control Release. 1993; 24: 201-208.
[33] Ozsoy Y, Gungor S, Cevher E. Nasal delivery of high molecular weight drugs. Molecules. 2009; 14: 3754-79.
[34] Hirai S, Yashiki T, Mima H. Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. Int J Pharm. 1981; 9: 173-84.
[35] Gordon G.S, Moses AC, Silver RD, Flier JS, Carey MC. Nasal absorption of insulin: Enhancement by hydrophobic bile salts. Proc Natl Acad Sci USA. 1985; 82: 7419-23.
[36] Uchida N, Maitani Y, Machida Y, Nakagaki M, Nagai T. Influence of bile salts on the permeability of insulin through the nasal mucosa of rabbits in comparison with dextran derivative. Int J Pharm. 1991; 74: 95-103.
[37] Dodla S, Velmurugan S. Buccal penetration enhancers-an review. Asian J Pharm Clin Res. 2013; 6: 39-47.
[38] Hoogstraate AJ, Senel S, Cullander C, Verhoef J, Junginger HE, Bodd HE. Effects of bile salts on transport rates and routes of FITC-labelled compounds across porcine buccal epithelium *in vitro*. J Control Release. 1996; 40: 211-21.
[39] Mahalingam R, Ravivarapu H, Redkar S, Li X, Jasti BR. Transbuccal delivery of 5-Aza-2′-deoxycytidine: Effects of drug concentration, buffer solution, and bile salts on permeation. AAPS Pharm Sci Technol. 2007; 8: E1-E6.
[40] Kim D, Ramasamy T, Choi JY, Kim JH, Yong CS, Kim JO, et al. The influence of bile salt on the chemotherapeutic response of docetaxelloaded thermosensitive micromicelles. Int J Nanomed. 2014; 9: 3815-24.