REVIEW ARTICLE

Recent advances on nano delivery of Helix mucus pharmacologically active components

Raffaele Conte*

Institute of Biosciences and Bio Resources, National Research Council, Via Pietro Castellino, Naples, Italy

Received 24 December 2015; revised 08 March 2016; accepted 16 May 2016; available online 02 July 2016

Abstract

Bioactive products from snail slime of "Helix" specie have potential applications in preventing and/or treating several human diseases and in cancer diagnosis. However, the poor pharmacokinetics characteristics of these natural compounds limit their use. Nanotechnology offers promising solutions for the enhanced formulation of these molecules through the synthesis of nanosized drug delivery systems. These vectors are characterized by facilitated transport across the biological barriers, enhanced bioavailability, targeted delivery and the capacity to protect sensitive compounds from biological and environmental degradation. Overall, this review focus on the description of bioactive natural substances derived from snails belonging to the genus helix and their successfully combinations with nanosized vectors.

Keywords: *Allantoin; Helix; Helicidine; Helix pomatia agglutinin; Nanoparticles; Nanotechnology; Solid lipid nanoparticles.*

How to cite this article

Conte R. Recent advances on nano delivery of Helix mucus pharmacologically active components. Int. J. Nano Dimens., 2016; 7(3): 181-185. DOI: 10.7508/ijnd.2016.03.001

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INTRODUCTION

Nanotechnology is the engineering of functional systems at molecular scale. It is a multidisciplinary field that employs techniques and tools from diverse disciplines, including biology, engineering, chemistry, and medicine [1]. Nanomedicine, a subfield of nanotechnology, is the application of nanomaterials to built nanodevices aimed to control human well-being. Nanomaterials are entities in the range of dimension from 1 to 100 nm that allow unique interactions with biological systems (Fig. 1a and 1b). Materials that are used in nanomedicine production include proteins, polymers, dendrimers, fatty acids, surfactants. The medical application of nanomaterials ranges from the use in diagnostics, e.g. colloids for radio pharmacy and contrast agents in magnetic resonance imaging, or in the manufacturing of nano devices for a targeted treatment. For example, nanosized drug delivery systems are designed to interact with cells and tissues with a high degree of functional specificity. These vectors are particularly suitable for this purpose because of their size and high loading capacities, their persistence at the target sites, their enhanced permeation and retention effects [2, 3].

* Corresponding Author Email: *raffaele.conte@ibbr.cnr.it*

Snails of Helix specie

Snails are members of the phylum Mollusca which contains at least 80,000 species and is the second largest phylum in the animal kingdom. Members of this group are found throughout the world. They are predominantly aquatic, but some are terrestrial. *Helix* snails belong to the class gastropoda, a group of land slugs (Fig. 2). Specifically, the main *Helix* species are:

Helix aspersa, also known as Cornu aspersum, native to the Mediterranean area. This gastropod is considered a pest in gardens and in agriculture, especially in regions where it has been introduced

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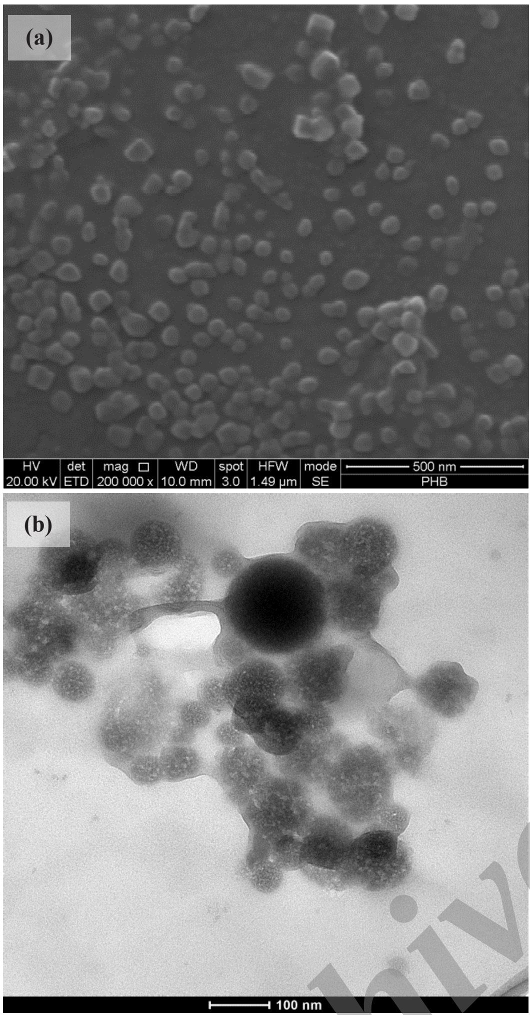

Fig. 1: a) SEM image of polyester nanoparticles
b) TEM image of polyester nanoparticles

Fig. 2: Helix species

accidentally. Phenotypically, adult exemplars have a hard shell of 25–40 mm in diameter and 25–35 mm high, with four or five whorls. The shell is brownish [4].

Helix Pomatia, known as escargot. This specie is highly prized as a food but, despite of this, it is rarely farmed. Helix pomatia is threatened by continuous habitat destructions and it is registered in the “IUCN Red List” and in the “European Red List of Non-marine Molluscs as Least Concern” [5]. Visually, the shell is white-brownish and has five to six whorls. The width of the shell is 30–50 mm and the height is 30–45 mm [4] (Fig. 3).

Helix lucorum, or Turkish Snail. The phenotype presents a white shell but almost entirely covered with broad brown bands. There are 4- 5 slightly convex and rapidly increasing whorls [4].

Helix vermiculata, or the “chocolate-band snail”. Its shell presents a whitish background color with darker colored bands or spots. The shell has 4-4.5 whorls. It is 22–32 mm width and 14–24 mm height [4].

History of the use of Helix in medicine

The land *helix* use in medicine is described by several authors since antiquity. Hippocrates proposed snail mucus against protocoele. Celse considered crude snails as healing substances and their boiled bodies as emollient materials. Pliny affirmed that *helix* pulp increases the speed of delivery and could be used to treat pain related to burns, abscesses, nosebleed, stomach pain and female scrofula [6]. Galien recommended snails against fetal hydrops [6]. In early eighteenth century, Ambroise Paré used snails against anthrax [6]. In the Universal Pharmacopoeia of Lemery is described the preparation of “snail water”, a distillate of crushed snails [6]. In 1808, George Tarenne published a book describing snail abilities to cure hernias [6]. The New Natural History Dictionary of 1817 indicated snails as remedies for throat diseases and as skin smoothing. Figuier, in 1840, promoted the use of snail mucilage for chest diseases, intestinal irritations and against tuberculosis [6]. Pharmaceutical formulations of snails were crude snails, sugar, tablets, syrup, snail paste, snail chocolate and snail ointment [6]. Moreover, Figuier mentioned the properties of hélicine, a transparent yellow oil, extracted from *Helix pomatia* and the other snails of the genus *Helix* [6]. Furthermore, the 1877 edition of Dorvault (a

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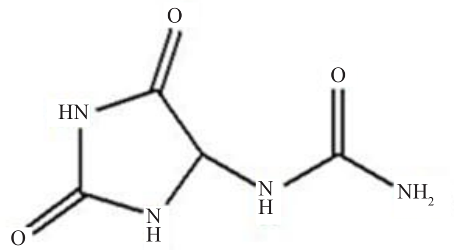

Fig. 3: Allantoin

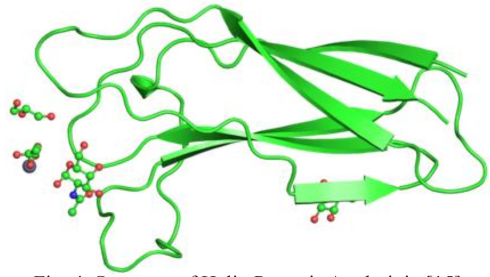

Fig. 4: Structure of Helix Pomatia Agglutinin [15]

French pharmaceutical reference book in 1800) mentioned several products based on snails: snail paste with donkey milk, “Quelquejeu” snail paste, “Figuier or de Mure” snail pectoral paste, “Figuier or de Mure” snail syrup, “Figuier” snail pectoral syrup, “Baron-Barthelemy” snail pectoral paste. A report of the same period listed the approved pharmaceutical formulations based on helicine and their indications against inflammations, cold, cough, bronchitis, catarrhs, asthma, various types of haemoptysis, tonsilitis, pharyngitis, hoarseness, sore throat, influenza, croup, whooping cough, nervous cough of children, lung diseases such as pneumonia and pulmonary phthisis, nervous stomach and intestinal cramps, gastritis, gastro-enteralgia, headaches coming from a pathological disorder of the stomach, measles, scarlet fever, small pox, erysipelas, irritation, weakening, and skin diseases [6]. Also in the 20th century the book Dorvault devoted an entire paragraph to snails. Besides, a review of snail therapeutic properties, in 1953, indicated the use of snail syrup for patients with chronic bronchitis, [7] emphasized the sedative properties of several formulations based on *Helix pomatia* for whooping cough and chronic bronchitis and reviewed the mucolytic and spasmolytic activities of *H.pomatia* mucus of in vitro [7].

Pharmacologic activities of Helix mucus components

The current preparation of helix mucus was described for the first time by Quevauviller in 1920. Briefly snails are soaked with 1% ww of sodium chloride solution. Mucus is then collected, settled, filtrated, concentrated and dried according to various processes (e.g. vacuum evaporation, freeze dried or spray dried). Finally, the resulting aqueous solutions is retained aseptically [7]. The obtained extract has several therapeutic properties. In cosmetic, it stimulates the formation of collagen, elastin and dermal components that repair the signs of photoaging. Moreover, it minimizes the damage generated by free radicals thanks to the presence proteins, high and low molecular weight hyaluronic acid and antioxidants [8]. In medicine, Allantoin (5- Ureidohydantoin), Helicidine, Helix pomatia agglutinin, Collagen, Elastin, Natural antibiotics and Glycolic acid (alfa-Hydroxyacetic acid) have important pharmacological actions (Fig. 4).

Allantoin is a chemical compound with formula $C_4H_6N_4O_3$. It is also called 5-ureidohydantoin or glyoxyldiureide. This substance is broadly used as active ingredient in over-the-counter cosmetics, thanks to its moisturizing and keratolytic effects, the ability to increase the water content of the extracellular matrix the capacity to enhance the desquamation of upper layers of dead skin cells and the aptitude to promote cell proliferation and wound healing. Moreover, Allantoin is a soothing, anti-irritant, and skin protectant agent by forming complexes with irritant and sensitizing substances. In vivo histological analyses reveal that a lotion with 5% allantoin ameliorates the wound healing process, by modulating the inflammatory response. Allantoin also promotes fibroblast proliferation and synthesis of the extracellular matrix [9]. Commercially, Allantoin is present in toothpaste, mouthwash, shampoos, lipsticks, anti-acne products, sun care products, clarifying lotions, cosmetic lotions, creams, and pharmaceutical products [10].

Helicidine is a mucoglycoprotein used as cough suppressant. The broncho-relaxant effect of helicidine is related with the release of E_2 prostaglandins and is inhibited by pre-treatment with indomethacine [11]. Helicidine relaxes airway smooth muscles, acting on tracheal smooth muscle or activating intermediary epithelial cells to release broncho-relaxant agents [11]. The effect of Helicidine on the trachea is explained by the release of the relaxant prostanoid, E_2 prostaglandin [11].

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Helix pomatia agglutinin (HPA) is a *N*-acetylgalactosamine (GalNAc) binding lectin with the physiologic function to protect snail fertilized eggs from bacteria. Besides it is part of the innate immunity system of the slugs. Helix pomatia agglutinin is used as a prognostic indicator for some cancers, such as breast, stomach and colon tumors. In fact, HPA fixation on histological preparations of these tissues is associated with ominous prognosis, indicating that HPA-associated glycoproteins are linked to metastasis of cancers [12-14].

α-linolenic acid is an hydrocarbon with a protective effect against cardiovascular diseases. α-linolenic acid prevents ventricular fibrillation and reduces platelet aggregation [16]. It derives from snails herbal nutrition. Other components are glycolic acid (GA), collagen and elastin. GA eliminates superficial dead skin cells and promotes their replacement with new corpuscles. It also enables cleansing of pilose follicles and favours the absorption of the other natural substances contained in the snail extract. Collagen and elastin are structural proteins that bond skin tissues and improve derma appearance. Collagen gives rigidity to connective tissue and organs while elastin confers stretching abilities. Specifically, the qualitative-quantitative chemical analysis of snail mucus reveal the presence of allantoin (0.3 - 0.5%), collagen (0.1 - 0.3%), glycolic acid (0,05 - 0.1%), lactic acid (0,05 - 0.1%), anti-protease (1.3 - 1.8%), vitamins, trace minerals and a high content of glycine, hydroxyproline, proline and glutamic acid [17].

Nanotechnology in the delivery of Helix slime components

In medicine, nanotechnology has the potential to improve the whole care process. There are diagnostic tools, imaging agents, drug delivery systems, pharmaceuticals, implants and tissue engineered constructs of nanoscopic scale available, aimed to make medical practice safer, less intrusive and more personalised [18]. In pharmacy, nanotechnology enhances bioavailability. This characteristic has a positive implication due to the fact that pharmacokinetics of snail components doesn't permit their optimal absorption. Nanotechnology responds to this problem through the incorporation of the drug into nanovectors. The synthesis of nano delivery systems enhance the efficacy and decrease side effects through a site-specific targeted delivery, help to increase the stability of drugs, and possess useful controlled release properties [3].

For example, Solid lipid nanoparticles (SLP) containing Allantoin are synthesized against cutaneous infections caused by yeasts and dermatophytes. Nanoencapsulation in SLP improves the antifungal activity of allantoin [19]. Nanoparticle suspensions is prepared using a high homogenization technique and the antifungal activity of the formulation is tested in vitro against the yeasts *Candida krusei* and *Candida parapsilosis*, and the fungal pathogens of human skin *Trichophyton rubrum* and *Microsporum canis*. SLP with allantoin have a diameter of 120 nm. Antifungal susceptibility shows a MIC90 of 7.8 µg/mL against *C. parapsilosis*, of 250 µg/mL against *C. krusei*, of 1.95 µg/mL against *T. rubrum* and of 1.95 µg/mL against *M. canis*. All these value are lower than the MIC90 of free allantoin. This result indicates that nanoencapsulation of Allantoin improves the antifungal activity, making this preparation a promising therapeutics for cutaneous infections caused by yeasts and dermatophytes [19]. For another, HPA-conjugated rare-earth doped fluoride nanocrystals are synthesized for the visualization of melanoma tumor [20]. The conjugation of HPA with nanocrystals is a process with several steps. Firstly, the hydrophobic $NaGdF_4$:Eu^{3+} (rare-earth doped fluoride) nanocrystals are obtained by a modified co-thermolysis method [21]. Then, they are functionalized to hydrophilic $NaGdF_4$:Eu^{3+} - COOH form through ligand exchange method [20]. Subsequently, the nanocrystal solution in toluene is mixed with another solution containing hydrophilic ligands such as trioctylphosphine oxide (TOPO) or meso-2,3-dimercaptosuccinic acid (DMSA).

The obtained reactive nanocrystals are conjugated with HPA dissolved in 2-(N-morpholino) ethanesulfonic acid (MES), 0.1M, pH 4.5 at 10 mg/mL, in presence of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride). Results show that HPA functionalized nanocrystals selectively recognizes regions of undifferentiated melanoblasts surrounding neoangiogenic foci inside melanoma tumor. HPA-$NaGdF_4$:Eu^{3+} are effectively used in fluorescent microscopy in visible and near infrared mode due to the fact that they possess high photostability. Further, they preserve intensive fluorescent signal at samples storage for at least 6 months [20].

CONCLUSION

The pharmacologic activities of *helix* slime components are broadly studied and recognized. The action mechanism of these substances is

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clear, making them appealing components of cosmetics and drugs. Despite of this, the poor pharmacokinetics of Allantoin, Helicidine and Helix pomatia agglutinin limit their therapeutic use. Current pharmaceutical exploration is on the synthesis of nano-scaled drug delivery systems able to enhance drug bioavailability. The examples of effective interactions between nanotechnology and *helix* mucus components show how this field of research is useful in both therapy and diagnosis. Overall, this review shows how this research is at an early stage and act as incitement for future developments.

ACKNOWLEDGMENTS

The author would like to thank colleagues from the nanotechnology group of Prof. Peluso at the "*Institute of Biosciences and BioResources*" of the Italian National Research Council (CNR), Naples, who provided insight and expertise that greatly assisted the research and the "*Electron microscopy facility* of the *Institute for Polymers, Composites and Biomaterials*", CNR, Pozzuoli for the acquired SEM and TEM images.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

[1] Gabor J. J. M. , Hornyak L., Dutta J., (2008), Fundamentals of nanotechnology. Boca Raton, FL: Taylor & Francis Group, CRC Press.

[2] Silva G. A., (2004), Introduction to nanotechnology and its applications to medicine. *Surg. Neurol.* 61: 216-220.

[3] Groneberg D. A., Giersig M., Welte T., Pison U., (2006), Nanoparticle-based diagnosis and therapy. *Curr. Drug. Targets.* 7: 643-648.

[4] Barker G. M., (2001), The biology of terrestrial molluscs. *CABI* Pub.

[5] Cuttelod A., (2011), European red list of non marine molluscs. *IUCN* Publications Services.

[6] Bonnemain B., (2005), Helix and drugs: Snails for western health care from antiquity to the present. *Evid Based Complement Alternat Med.* 2: 25-28.

[7] Quevauviller A., Garcet S., (1953), The mucus of Helix pomatia L.; preparation, composition, therapeutic and pharmacodynamic properties, biologic assay. *Rev. Pathol. Gen. Physiol. Clin.* 53: 653-658.

[8] El Mubarak M. A., Lamari F. N., Kontoyannis C., (2013), Simultaneous determination of allantoin and glycolic acid in snail mucus and cosmetic creams with high performance liquid chromatography and ultraviolet detection. *J. Chromatogr. A.* 1322: 49-53.

[9] Ulhôa Araújo L., Grabe-Guimarães A., Furtado Mosqueira V. C., Martins Carneiro C., Silva-Barcellos N. M., (2010), Profile of wound healing process induced by allantoin. *Acta Cirúrgica. Brasileira.* 25: 7-10.

[10] Thornfeldt C., (2005), Cosmeceuticals Containing Herbs: Fact, Fiction, and Future. *Dermatologic. Surg.* 31: 145-149.

[11] Pons F., Michelot R., Mayer M., Frossard N., (2008), The bronchorelaxant effect of helicidine, a Helix pomatia extract, Involves prostaglandin E2 release. *Pharmaceutical Biology.* 36: 7-11.

[12] Dwek M.V., Streets A. J., Brooks S. A., Adam E., Titcomb A., Woodside J. V., (2001), Helix pomatia agglutinin lectin-binding oligosaccharides of aggressive breast cancer. *Int. J. Cancer.* 95: 155-159.

[13] Brooks S. A., Leathem A. J., (1991), Prediction of lymph node involvement in breast cancer by detection of altered glycosylation in the primary tumour. *Lancet.* 338 : 71-74.

[14] Rambaruth N. D., Greenwell P., Dwek M. V., (2012), The lectin Helix pomatia agglutinin recognizes O-GlcNAc containing glycoproteins in human breast cancer. *Glycobiology.* 22: 839-848.

[15] Sanchez J. F., Lescar J., Chazalet V., Audfray A., Gagnon J., Alvarez R., (2006), Biochemical and structural analysis of Helix pomatia agglutinin. A hexameric lectin with a novel fold. *J. Biol. Chem.* 281: 20171-20180.

[16] Pan A., Chen M., Chowdhury R., Wu J. H., Sun Q., Campos H., (2012), Alpha-Linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. Am. *J. Clin. Nutr.* 96: 1262-1273.

[17] Conte R., (2015), Heliciculture: Purpose and economic perspectives in the European community. *IST Journal.* Spring 2015.

[18] Kubik T., Bogunia-Kubik K., Sugisaka M., (2005), Nanotechnology on duty in medical applications. *Curr. Pharmac. Biotechnol.* 6: 17-22.

[19] Svetlichny G., Kulkamp-Guerreiro I. C., Cunha S. L., Silva F. E., Bueno K., Pohlmann A. R., (2015), Solid lipid nanoparticles containing copaiba oil and allantoin: development and role of nanoencapsulation on the antifungal activity. *Pharmazie.* 70: 155-164.

[20] Dumych T., Lutsyk M., Banski M., Yashchenko A., Sojka B., Horbay R., (2014), Visualization of melanoma tumor with lectin-conjugated rare-earth doped fluoride nanocrystals. *Croatian Medical Journal.* 55: 186-194.

[21] Banski M., Podhorodecki A., Misiewicz J., (2013), $NaYF_4$ nanocrystals with TOPO ligands: Synthesis-dependent structural and luminescent properties. *Phys. Chem. Chem. Phys.* 15: 19232-19241.

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