Anti-inflammatory Action of Toothpastes Containing Betulin Nanocapsules

DANIELA JUMANCA[1], ATENA GALUSCAN[1*], ANGELA CODRUTA PODARIU[1], FLORIN BORCAN[2], KAMEL EARAR[3*]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, Faculty of Dentistry Medicine, Department I, 14A Tudor Vladimirescu Str., 300173, Timisoara, Romania

[2] "Victor Babes" University of Medicine and Pharmacy Timisoara, Faculty of Pharmacy, Department II, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[3]"Dunarea de Jos" University of Galati, Faculty of Medicine and Pharmacy 35, A.I.Cuza Str, 80010, Galati, Romania

Nanomedicine may be defined as an application of nanotechnology in the field of health, healthcare, diagnosis and treatment of diseases, with the purpose of maintaining and/or improving populational health. This is also applicable to dental medicine. The aim of this study was to design a toothpaste based on natural ingredients, with antiinflammatory and antibacterial proprieties which could be used to decrease the degree of gingival inflammation. In order to increase the biodisponibility of the therapeutic substance i.e. birch sap extract, it has been incorporated into polyester urethane nanocapsules thus being more easily transported to its action site. After the invention patent was published for the composition of this toothpaste, it was tested on 32 volunteers. After using the toothpaste for two weeks, the gingival inflammation index (GI) decreased from an average of 2.3 to 1.028.

Keywords: nanocapsules, betulin, gingival inflammation

A healthy oral cavity, without dental destruction and soft tissue inflammation is the desire of any educated person nowadays. Despite preventive measures, in certain conditions inflammation of the gum or of the other soft tissues may occur even in the most careful individuals. Gingival inflammation must be diagnosed and treated from the very first symptoms in order to avoid the evolution towards periodontitis and further to parodontosis, by the impairment and degenerescence of the tooth supporting tissues. Attention must also be paid to stomatitis which is the inflammation of the oral mucosa and may affect the entire oral cavity and also to oral blisters which are extremely painful and cause feeding and speech difficulties and even the inflammation of local and regional lymph nodes.

In order to maintain the balance of the oral ecosystem, together with food hygiene and hygiene measures, the use of natural products protecting the hard dental tissues and preventing soft tissue inflammation is highly recommended. As the main cause of periodontal inflammatory diseases are the excessive accumulation of microorganisms in the shape of soft and mineralised dental deposits, the safest and most effective prophylactic and therapeutic method for inflammation is the removal of these deposits by mechanical methods. Because during the removal process mechanical trauma may occur in the inflamed tissues which are contaminated with pathogens (possibly leading to bacteriaemia which is an extremely dangerous complication for persons with allergic-infectious disturbances of the cardiovascular system), the procedure is recommended after the acute inflammation is treated or during its remission, with extreme attention. Upon marked gingival inflammation with abundant purulent exudate, prior to scaling the treatment of acute inflammation with antiseptic products is recommended.

For this reason, toothpastes containing natural anti-inflammatory and antibacterial substances are recommended. The inclusion of biologically active substances in polyesther urethane nanocapsules is a novel approach in the field of nanomedicine, these being a transdermic transportation vehicle for therapeutic substances in anti-inflammatory and antibacterial toothpastes; thus, the biodisponibility of the active substance is increased.

Nanomedicine may be defined as an application of nanotechnology in the field of health, healthcare, disease diagnosis and treatment, for the purpose of maintaining and/or improving populational health using molecular knowledge on the human organism, as well as nanometric instruments/structures [1]. For this, physical, chemical and biologic features of materials at nanometric scale are used, these features being often new or improved, and the resulting nanostructures (nanoparticles or nanodevices) sharing the same dimensions with the biological entities which enables them to more rapidly interact at biomolecular level, both at the surface and inside the cells [2]. Some of the structures and devices used by nanomedicine are: nanoparticles, nanotubes, liposomes, fullerenes and nanodevices.

Nanoparticles were created to improve the biodisponibility in the field of pharmacology, knowing that the biodisponibility of various forms of drugs has been a major limitation for obtaining new, more effective medicines; a good example is the low biodisponibility in the case of RNA interference therapies. Due to very low dimensions, nanoparticles may easily enter cells, being a vehicle for various drugs and they may also be directed to certain target cells; thus, the danger of toxicity is eliminated and the effectiveness of the treatment increases [3]. They may transport medicines or genetic material to the internal cell environment without causing side effects as nanoparticles only become active after reaching the final destination. Also, overdose related phenomena are prevented, drug intoxication being thus avoided. For nanoparticles, as for drugs, efficacy as well as safe administration must be evaluated in parallel.

* *email: atedent@yahoo.com; Tel.: 0040256204950; erar_dr.kamel@yahoo.com*

Experimental part

During recent years, numerous studies on the role of nanometric substances in dental tissues were conducted [4]. It has been proved that toothpastes containing nano-hydroxyapatite lead to a more pronounced bovine dentine and enamel remineralisation as compared to toothpastes containing fluorinated amines [5]. Also, action may be taken upon periodontal tissues by including anti-inflammatory substances into nanoparticles which act as transdermic transportation vehicles.

In order to prevent and reduce already existing gingival inflammation, we designed a toothpaste with natural ingredients, with antiinflammatory and antibacterial properties. In order to increase the biodisponibility of the therapeutic substance i.e. birch sap extract, this was included into polyesther-urethane nanocapsules, thus becoming more easily transported to the action site.

The composition of this toothpaste has been recorded as an invention patent by the Romanian State Office for Inventions and Trademarks (OSIM) under the number 127805/28.02.2014.

Materials and methods

We chose birch sap extract, betulin, as therapeutic substance, due to its worldwide known therapeutic properties. Birch leaves contain: flavonoids (especially rutosides and hyperoxides), polyphenols, tannins, allantoin, organic acids (phenolic, caffeic, chlorogenic), methyl-salicylate, leucoanthocyanins, triterpenic alcohols (betuloprenol, betulenol), etheric oil, vitamin C and minerals [6].

We used birch sap which is rich in betulin and betulinic acid, widely used in the pharmaceutic industry [7]. PPS-powder betulin was extracted and then incorporated in nanocapsules.

Betulin is known to possess antiinflammatory properties and to be active in preventing tumoral growth [8] and the inclusion in nanocapsules increases its curative properties.

The polyesther urethane nanotubes were synthesised in the Department II of the Faculty of Pharmacy of the University of Medicine and Prahmacy Timisoara, based upon a polycondensation technique at the interface of two phases combined with spontaneous emulsionation (fig. 1).

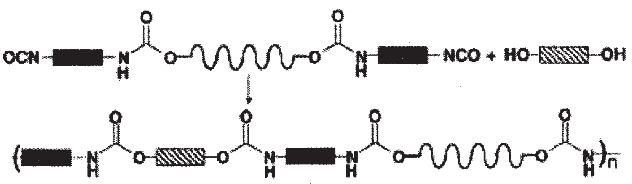

Fig. 1. The reaction of polyadition

After including the betulin extract in the synthesised nanotubes, scanning electron microscopy (SEM) was performed. Based upon the obtained images (fig. 2), we concluded that introducing the active substance does not influence the aspect and dimension of nanotubes.

Toothpastes are known to usually contain a wide variety of components with well defined functions, namely:

- tensioactive agents, usually anions of the class of alkaline soaps, sodium alkyl polyether sulphonates or sodium alkyl sulphonates with liniar hydrocarbonate structure;
- microcrystalline or amorphic abrasive and polishing agents such as magnesium, calcium or aluminium insoluble salts (carbonates, sulphates, pyrophosphates, silicates), silicium, aluminium, titan or calcium oxides or peroxides;

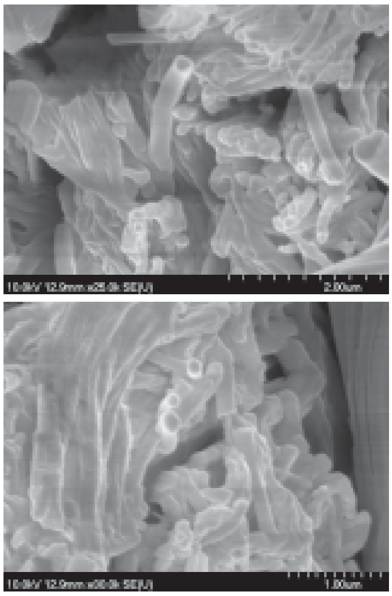

Fig. 2. The SEM image of the probe at a scale of 2 μm and 1 μm

- organic gellification/bounding agents such as carboxymethyl cellulose, carrageenans, xanthan, polyvinyl alcohol, polyethylene glycol, hydroxyethyl or hydroxypropyl cellulose, polyols, vinyl polymers such as polyethylene, polypropylene, vinyl polychlorure, polyamide/nylon, silicone polymers, fats and waxes, or anorganic hydrosoluble substances such as alkaline chlorides, sodium citrate, succinate, tartrate, anorganic insoluble compounds such as colloidal silica, magnesium and aluminium silicates, gellified aluminium hydroxide, bentonite or organic/ anorganic mixtures such as carrageenans and synthetic or amorphic sillicium dioxide;
- polyol type moisturizers such as glycerine, sorbitol, xylitol, lactitol, polyethylene glycol;
- conservants, usually synthetic products such as: aliphatic (alcohols), aromatic (polyhalogenated phenols) hydroxylic compounds, sodium benzoate, sodium salicylate or formaldehide;
- natural or synthetic nonfermenting sweeteners of the polyol class: glycerine, sorbitol, glucides or saccharine;
- fluoride containing combinations, as healing agents: sodium and potasium fluorides, zinc fluorides, fluorophosphates, aminofluorides; or without fluoride such as sodium trimethylphosphate, hydroxyphosphates;
- antimicrobial substances: chlorhexidine, metronidazol, quaternary amonium salts, bis-guanidin, halogenated compounds with bis-phenolic structure, Trichloro-2'-hydroxy-diphenyl-ether, known as triclosan [9]. The chemical inhibitors act according to the following objectives: to disrupt the metabolism of the bacterial plaque and its iochemical mechanisms or to act upon the enamel structure in order to make it more resistant to the action of the bacterial plaque [10].
- antiinflammatory agents: ibuprofen, aspirin, indometacin;
- vitamins such as A, C, E; colorants and enzymes such as proteases [11].

For our toothpaste we chose the following composition: low content of anionic tensioactive substances, from fatty acids, vegetal abietic acid and lauryl sodium diethylethersulphate; carboxymethylcellulose-walocel gellification agent; natural waxes with adhesive properties; precipitated calcium silicate in micro- and nanometric mixture as cleaning agent; acacia honey as sweetener, PPS-powder betulin extract incorporated in nanocapsules, as vegetal bacteriostatic and anti-inflammatory agent, and water up to 100%. The proportions of components were within the following intervals: 5-11% anionic tensioactive substances, 0.7-3.5% carboxymethylcellulose-walocel gellification agent, 0.1-0.3% natural waxes with adhesive properties, 7-10% micro and nanometrically precipitated calcium silicate as cleaning and nano-sealing agent, 7-10% acacia honey as sweetener, 0.2-2% betulin PPS/nanocapsules as bacteriostatic and anti-inflammatory agent and a high water content of 65-80%, with a good stability in time, without segregation of solid or organic and watery phases.

The percent distribution of the main ingredients used for toothpaste formulation shows the following: 0.2….20% tensioactive substances from natural or synthetic precursors, 5….50% polishing agents, 0.1….8% gellification or mixture of organic or anorganic gellification/bounding agents, 2….70% moisturizer, 5…70% water. All the other types of ingredients with specific functions as conservants, sweeteners, flavouring agents, anticarious substances, antimicrobials, anti-inflammatory agents, vitamins, enzymes, colorants represent a total of 1….25%.

The toothpaste designed by us has a series of advantages over other toothpastes, namely:

- low content of tensioactives and additives which lowers the fabrication costs and the impact on the oral mucosa;
- all the organic components (tensioactive substances, moisturizer, gellification and antiinflammatory agents) are biocompatible with the human orgamnism and biodegradable;
- the cleaning agent i.e. the precipitated calcium silicate, in a mixture of micro and nanoparticles, also acts as nano-sealant of enamel micro fissures;
- its gellification and stabilisation system contains a certain type of carboxymethylcellulose-walocel, natural waxes of colophonium with adhesive properties, i.e. sodium salt of the abietic acid, the latter acting both as a tensioactive as well as a heterocyclic substance which renders adhesive properties favouring the stability of the gel, thus helping to avoid phase segregation, with a beneficial impact on the increase of the water content around the ideal value of 70%;
- bacteriostatic and antiinflammatory agents are vegetal biologically active products: PPS-powder betulin extract incorporated into nanocapsules.

The therapeutic qualities of the toothpaste were checked in two ways.

Before submitting the application for the invention patent, at the Faculty of Pharmacy (UMPT), the toxicological analysis was done and the results were communicated in the analysis report 006/11.10.2011. BALB/c mice were used (two mice for each group). The work protocol followed all National Institute of Animal Health (NIAH) rules: animals were maintained during the experiment in standard conditions offered by the Biobase of University of Medicine and Pharmacy Timisoara: 12 hours light-dark cycle, food and water *ad libidum*, temperature 23°C, humidity above 50%. It was obtained the permission from the Ethical Commission on Animal Experiments of the "Victor Babeş" University of Medicine and Pharmacy Timişoara. All requirements of the equipment manufacturer, (Germany), have been met: room temperature (20-23°C); humidity (40-60%); the subjects skin was shaved one day before measurements (where it was necessary); all the measurements were done in the same moment of the day by the same operator. All the measurements on the mice skin were carried out with a Multiprobe Adapter System (MPA5) from Germany, equipped with a Tewameter® TM300 probe, a skin-*p*H-meter® PH905 probe, a Sebumeter® SM815 probe, a Mexameter® MX18 probe and a Corneometer® CM825 probe. No sebum was detected in these determinations and this was due to age of mice. Measurements were repeated three times on every mouse and average values were used. Data were analysed using one-way ANOVA and Tukey's post hoc analysis.

After the publication of the invention patent, we tested the anti-inflammatory properties *in vivo*. A group of 32 persons with gingivitis volunteered to use the betulin nanocapsules toothpaste for two weeks instead of their currently used product. The written informed consent was obtained from all participants who also signed their consent to follow the instructions regarding dental brushing.

In order to determine the degree of dental inflammation we used the gingival index of Loe and Silness (GI) which scores as follows: each gingival dental unit is scored (vestibular, oral, mesial and distal sectors). Scoring starts from 0 to 3 depending on inflammation, colour change and bleeding [12]:

-0 = normal gingiva;

-1 = slight inflammation: slight change in colour and structure;

-2 = moderate inflammation: moderate shine, erythema, edema and hypertrophy; bleeding upon probing;

-3 = pronounced inflammation: marked erythema and hypertrophy; tendency to spontaneous bleeding; ulceration

In this study we only included the vestibular sector of the upper and lower frontal teeth between the canines i.e. a total of 12 teeth per patient. Measurements were done at the start and at the end of the treatment for comparison. At the beginning of the treatment the average gingival inflammation index was 2.3; after two weeks of using the toothpaste with betulin nanocapsules the average decreased to 1.028.

For each patient, the average gingival inflammation index was calculated at the beginning and at the end of the treatment, in order to be able to quantify the anti-inflammatory effect of the toothpaste. The obtained values may be seen in the following graph in which the upper line represents the initial values of the gingival inflammation index and the lower line represents the final values of the gingival inflammation index.

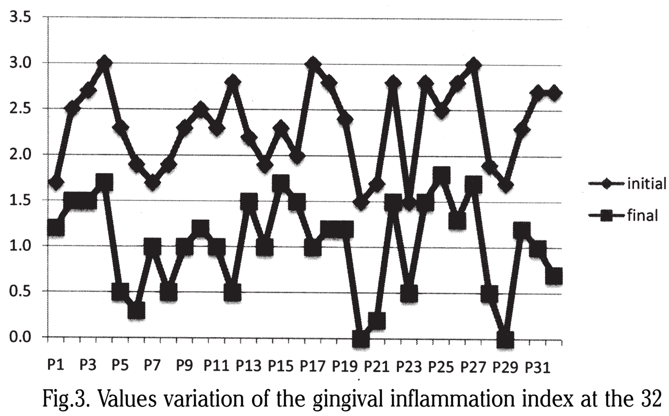

Fig.3. Values variation of the gingival inflammation index at the 32 patients; with red the initial values and with blue the final situation.

At the beginning of the treatment the average gingival inflammation index was 2.3 and after two weeks of using the toothpaste with betulin nanocapsules, the average decreased to 1.028.

Conclusions

By using nanotechnology in the field of medicine, nanocapsules or nanotubes may be obtained which can interact more rapidly at biomollecular level, both on the cell surface as well as inside the cells.

Nanomedicine also finds applicability in dental medicine [13-15], an example being the inclusion of betulin powder in toothpaste using nanocapsules. Thus, the bacteriostatic and anti-inflammatory capacity of this vegetal product is enhanced, nanocapsules being a means for transdermic transportation of the therapeutic substance contained in the toothpaste.

The use of the toothpaste which is recorded as an invention patent by OSIM under the number 127805/ 28.02.2014 led to a decrease of the average gingival inflammation index from 2.3 to 1.028.

References

1. *** http:// www.snspms.ro Nanomedicina – medicina viitorului. Dr. Marius Ciutan, Dr. Carmen Sasu, Monika Skiba.

2.***European Technology Platform on NanoMedicine, *Nanotechnology for Health - Vision Paper*

3.*** lhttp://biotech.about.com/gi/o.htm?zi=1/XJ&zTi=1&sdn= biotech&cdn= money&tm=32&gps=200_59_985_517&f=00&su= p649.3.336.ip_&tt=2&bt=1&bts=1&zu=http%3A//www.nano-biology.net/

4. FILIP L., NEDELCU I.A., SONMEZ M., ANDRONESCU E., Rev. Chim. (Bucharest), **65**, no. 2, 2014, p. 521

5. TSCHOPPE P, DANIELA L. ZANDIM P. M., Kielbassa A.M.: Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. Journal of Dentistry 39 (2011) 430–437

6.*** http://ziarullumina.ro/sites/default/files/articol/2009-07/ 95526input_file0094110.jpg

7. *** http://ro.wikipedia.org/wiki/Mesteacăn

8. *** www.umfcluj.ro/ro/sustinute-ro/category/9-rezumate-2013

9. GALUSCAN A., JUMNCA D., BORCAN F., SOICA C.M., IONESCU D., RUSU L.C., CRAINICEANU Z., Rev. Chim. (Bucharest), **65**, nr 2, 2014, 190

10. GALUSCAN A., JUMNCA D., VASILE L., PODARIU A.C., ARDELEAN L., RUSU L.C., Rev. Chim. (Bucharest), **63**, no. 7, 2012, p. 707

11. PODARIU A. C., JUMANCA D., GALUSCAN A., VACARU R., MUNTEAN R.: Tratat de Prevenţie Oro-dentară. Editura Waldpress Timişoara 2003

12. JUMANCA D., PODARIU A.C., GALUSCAN A., OANCEA R., POPOVICI R., SAVA ROSIANU R.: Preventie oro-dentara. Editura Marineasa, Timisoara 2010.

13.PATIL M., METHA D.S., GUVVA S.: Future impact of nanotechnology on medicine and dentistry. Journal of Indian Society of Periodontology 2008 May-Aug; 12(2); 34-40.

14. MITSIADIS TA, WOLOSZYK A, JIMÉNEZ-ROJO L.: Nanodentistry: combining nanostructured materials and stem cells for dental tissue regeneration. Nanomedicine (Lond). 2012 Nov; 7(11): 1743-1753.

15. KANAPARTHY R., KANAPARTHY A.: The changing face of dentistry: nanotechnology. International Joulnal of Nanomedicine. Dove Press Journal, 2011:6 2799–2804

Manuscript received: 25.02.2014