International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 481-495 www.ijprajournal.com ISSN: 2249-7781

A detailed review on role and mechanism of Enzymes used in mouth care product

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Submitted: 15-01-2022 Accepted: 27-01-2022

ABSTRACT:
In a review,we study enzymes, their chemical action on bacteria, and various actions including biological roles, inhibition, etc.The enzyme is not only present in mouth care products but they are itself present in the human body where they are situated at different locations with different enzymatic activities. Some enzymes are present in organic products such as milk in form of protein, but all enzymes are proteins; not all proteins are an enzyme.
Enzymes are present in various mouth care products and are an important element of the nonspecific immune response involved in maintaining oral health. The main role of an enzyme such as Lactoperoxidase is to oxidize salivary thiocyanate ions (SCN−) in the presence of hydrogen peroxide (H2O2) to products that exhibit antimicrobial activity. LPO derived from bovine milk has found an application in food, cosmetics, and medical industries due to its structural and functional similarity to the human enzyme. Oral hygiene products enriched with the LPO system constitute an alternative to the classic fluoride caries prophylaxis
The enzyme can support host immune responses, and thus maintain oral health. This study aimed to investigate gingival health and the plaque-reducing effects of enzyme-containing nine in mouth care products. A laboratory study tested the antimicrobial potential of different enzyme-containing toothpaste formulations, mouthwashes, and various mouth care products.
Keywords: Lactoferrin, dentifrice**,** Lactoperoxidase, Inhibition of microbes, Serratiopeptidase, Role of Enzyme on the drug.

I. INTRODUCTION:-

This review aimed to present the latest knowledge about the physiological role of human salivary lactoperoxidase and Lactoferrin. The role of reactivators and inhibitors of LPO, in recent years,has been investigated and ithas been reported together with the use of nanoparticles in order to stabilize and improve the enzyme activity.It assists tocontrol the iron which isabsorbed through the intestinal tract into the body. Lactoferrin also helps to protect against infections from bacteria, viruses, and fungi. Lactoferrin seems to decrease the growth rate of bacteria by starving them of nutrients. It also destroys the walls around the bacteria at the cellular level.

Among various bacterial species, many are estimated to existin the human oral cavity; these are disease-causingbacteria that can lead to conditions such as dental caries, gingivitis, and oral malodor. Various studies and researchhave indicated a various connections between periodontal disease and various systemic diseases such as diabetes, heart disease, cerebrovascular disease, pneumonia, rheumatoid arthritis, and kidney disease, periodontal pathogens may be involved(suggested) in the mechanisms underlying these conditions. As such, emphasis has been placed on the prevention of periodontal disease and maintenance of healthy oral function as part of overall health in the second term of the NHPM in the 21st century (Health Japan 21, the second term), which was initiated in April 2012-2013. However, a Survey of Dental Diseases (Ministry of Health, Labour and Welfare, 2011) revealed that periodontal disease more than 80% of peopleaged between 30 and 70 years, hence suggesting addressing such issues. Although the number of teeth retained by older persons has been increasing, with every 1 in 4 individuals at age of 80 are still having at least 20 teeth and there has also been an increase in the frequency and intensity of the periodontal disease. Additional care is recommended to maintain oral health the older people since oral hygiene decline/reduces as saliva production decreases with age. (LF) Lactoferrin and (LPO) lactoperoxidase are the factors in exocrine secretions (defense), including milk and saliva including the defensive factors and their concentrations in milk and saliva [primariliy] (5–9). LF has various properties such as antibacterial activity against pathogens (including periodontal

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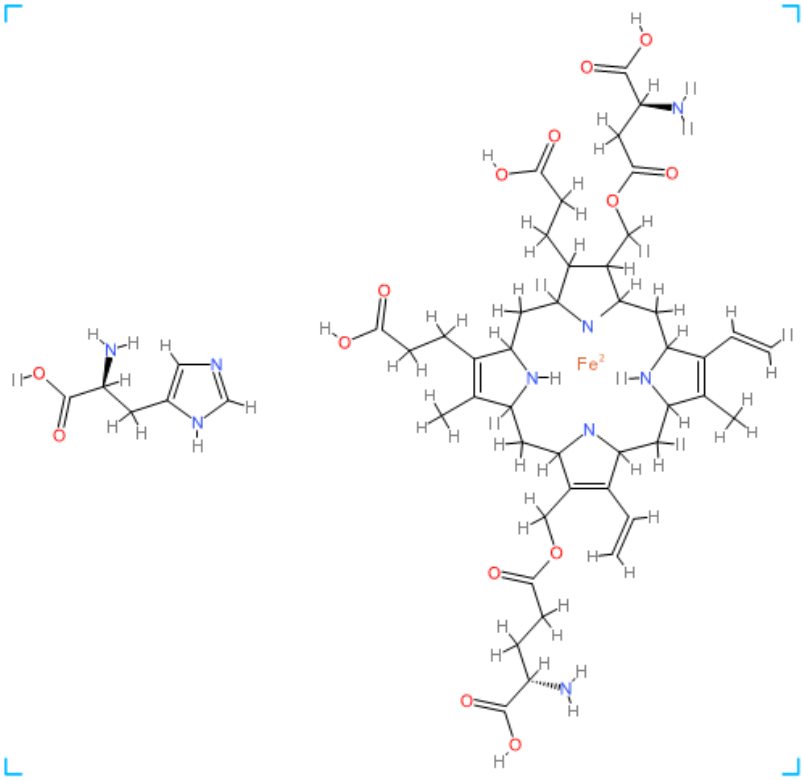
bacteria), anti-inflammatory activity, and anti-biofilm activity, and alsoa property to bind with iron (glycoprotein) (10). LPO (heme-binding glycoprotein) shows strong antibacterial activity against various pathogens including periodontal bacteria by catalyzing the generation of hypothiocyanite (OSCN−) from seventhiocyanates(SCN−) and hydrogen peroxide in saliva (11), which are an antimicrobial cascade recognized as the LPO system. It has been reported glucose oxidase (GO) acts asa source of hydrogen peroxide (12). Based on these reports, LF and LPO are to be important for the maintenance of oral hygiene in the oral cavity (12–14).Lactoperoxidase (LPO) is a glycoprotein thatis found in milk, saliva, and other forms of exocrine secretions (16).

It catalyzes the hydrogen peroxide-dependent oxidation of thiocyanate (SCN–) to hypothiocyanite (OSCN–), which is a potent antimicrobial agent which is a factor tworksworkagainst bacteria, fungi, and viruses (16).The Lactoperoxidase system is also knownas the antimicrobial system. OSCN–reacts with microbial thiol groups and inhibitsvarious functions such as glycolysis (17), the membrane transport of sugars and amino acids (18),respiration (19), and the urease activity of Helicobacter pylori.

Ion Dentistry is the branch of medical science dealing with the investigation, treatment, and prophylaxis of the ailments of teeth and oral cavity. Oral tooth related problems(like bleeding in gum,Tooth decay and.etc) have increased in high frequency as one of the effects of the modern lifestyle and so dental visits have become common nowadays. Although, sometimes dental includes operative or surgical procedures mostly. However,the majority of doctorsstill rely on medications for a person's health, as part of preoperative/postoperative management. These include drugs like analgesics, anesthesia, dietary supplements, antibiotics, steroids or anti-anxiety, etc. Presently to obtain better anti-inflammatory or analgesic effects of medicines;enzymes are been globally used by dentistry which is discussed in this study and we are going to give a review on some of the few enzymes includingSerratiopeptidase, chymotrypsin, etc.

Structure of Lactoperoxidase:



International Journal of Pharmaceutical Research and Applications
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Dentifrices with the LPO System

Available dentifrices comprising the LPO system are usually made of lactoperoxidase, potassium thiocyanate, and a system used for generating hydrogen peroxidases such as glucose oxidase (GOx), xanthine oxidase (XO), or lactose oxidase. Additionally, these preparations are enhanced with other nonspecific bioactive components such as lysozyme, lactoferrin, or antigen-specific immunoglobulins from bovine milk. Lactoferrin is a glycoprotein belonging to the transferrin family that can bind iron. Its antibacterial properties are due to the ability of iron sequestration that makes it inaccessible to many species of bacteria, including cariogenic S. mutants [69]. In addition, it interacts with Gram-negative lipopolysaccharide, releasing them into the environment [70]. Lysozyme, or muramidase, is a strongly cationic antibacterial protein found in many body fluids [71]. Its activity is based on the hydrolysis of glglycosidiconds in thepeptidoglycanof bacterial cell walls [72] and increase in cell membrane permeability, as well as inhibiting biofilmdevelopment [73]. The mixture of naturally occurring proteins which are found in oral hygiene productsincreases their mutual effects and supports the antimicrobial protection system of the host, resulting in improved gingival health parameters and changing the ecology of the bacterial flora, i.e., the increase of the share of species related to oral health. Protein components present in pastes might be depositedafter brushing on the surface of the renewing pellicle. In the case of LPO, its activity was detectable after 40 min from the formation of the acquired pellicle for three tested enzymatic pastes [74]. There are various forms of marketed products available for applications of oral hygiene containing LPO (such as lozenges, gels, foams, and mouthwashes) and hence it is important to know that they develop a supplement to teeth brushing and hence can be easily excluded during everyday oral care routine while providing a convenient alternative to toothpaste for use outside the home. Currently, caries prophylaxis involves the use of classic fluoride preparations [75, 76] and intensively studied non-fluoride methods including chlorhexidine [77], probiotics [78], xylitol [77, 79], triclosan [80], CPP-ACP [81], and proteins of natural origin. Dentifrices along with antibacterial proteins such as LPO can be a substitute to common fluoride preparations. The currently recommended form of caries prophylaxis involves the use of pastes and other products containing fluoride [82]. Despite the wide prevalence of this prophylaxis, caries and periodontal disease are still mmamajorobproblems any age group. The utilization of varnishes and gels witha high concentration of F− and low pH (3.5–5) may lead to local inhibition of OSCN− production bythe LPO system, whereas fluoride present in enzymatic pastes do not cause any inhibitory effect on the LPO system due to pH > 5.5 [83]. A mild surfactant, stearyl ethoxylate 30, is used in pastes including the LPO system rather than commonly used sodium lauryl sulfate (SLS). SLS isshownleading to reduction of the protective effect of mucin of the mucous membrane, desquamation of oral epithelium, and contributes to the development of irritation [84, 85] and gingival sloughing [84]. Chlorhexidine (CHX) which is used in rinses and gels as one of the alternatives to reduce plaque in gingivitiswhich is specified by a strong bactericidal effect because of its positive charge which allows adsorption on the mucous surface, teeth, biofilm components which includes bacteria, EPS, and glycoproteins.

Lactoperoxi dasesystem

The reaction mixturesbelonging to the LPO system contain 4.21 units/ml of LPO which are purified from bovine milk (FrieslandCampina Domo) along with 0.4 units/ml of GO which is purified from Penicillium chrysogenum mixed with 1.55 mm glucose, and 0.66 mm sodium thiocyanate. The reaction mixture was prepared in an Anaerobic environment because the reaction through GO required dissolved oxygen. The reaction mixture in 40 mm phosphate buffer (pH 7.7) was incubated at 37°C for 10 to 30 min without adding 14bacteria and the concentration of OSCN– was measured by monitoring the reaction with 5-thio-2-nitrobezonic acid [20].

Chemical Mechanism of Action

The first step in the cycle of lactoperoxidase activity is the transition state which is from the native form to Compound I by the two-electron oxidation in the presence of hydrogen peroxide (alternatively, organic peroxides), which is reduced to water [21]

The creation of compound can be represented by this reaction

LPO (native form) + H_2O_2 → LPO (Compound I) + H_2O

Inhibitors:

Numerous substances acting through three main mechanisms can inhibit the biocidal activity

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of LPO. The first is the pre-enzymatic mechanism which consists in competing for access to substrates. This group of inhibitors may include physiological salivary components such as myeloperoxidase (whose concentration increases in periodontitis [22]) and catalase [23] competing for hydrogen peroxide with LPO, or uric acid present in saliva at high concentration and competing with SCN^- ions [24]. The second group of inhibitors is substances interacting with the enzyme molecule, e.g., cyanides, azides, or thiourea [25]. The third post enzymatic mechanism of inhibition is associated with the reduction of reactive lactoperoxidase products. This group contains compounds consisting thiol groups, such as glutathione which are present in saliva (whose concentration increases in caries [26]), which may cause weakening of the LPO system. This group also includes NADH-OSCN oxidoreductase, which are responsible for the $OSCN^-$ breakdown to SCN^- [27].

Commonly used drugs, e.g., some indazoles [28], carbidopa [29], salicylic acid and some other phenolic acids [30], propofol [31], some antibiotics, and some corticosteroids [32] also exert the inhibitory effect on LPO.

Biological Role of the LPO System:

The key products of the lactoperoxidase system are hypothiocyanous acid (HOSCN) and hypothiocyanite ions ($OSCN^-$) formed during the oxidation of thiocyanates [33]. Both the products belongs to dynamic equilibrium state, which transforms into one another. The ratio of the dissociated form ($OSCN^-$) to the non-dissociated one (HOSCN) depends on the pH of the environment [34]. Hypothiocyanitehas a strong selective oxidizing activity on the thiol moieties of key enzymes in the course of glycolysis in microorganisms[35]. The mechanism of inactivation: a transient product (R-S-SCN) are produced by thiol group (R-SH) oxidation, which then breaks down to release a hydroxyl this moiety (R-S-OH); thiocyanate ions that can be Re-oxidized and undergo further reactions with thiol groups [35] It can be summarized by the following reactions.

$R\text{-}SH + OSCN^- \rightarrow R\text{-}S\text{-}SCN + OH^-$

$R\text{-}S\text{-}SCN + H_2O \rightarrow R\text{-}S\text{-}OH + SCN^- + H^+$

Still, the inhibitory effect of $OSCN^-$ ions causing growth of microorganisms may be transformable if these ions are excluded from the environment and leading to high concentration of reducing agents such as glutathione or NAD (P) Hpresent in the cell [35]. The appearance of intracellular NADH: hypothiocyanite oxidoreductase in commensally Streptococcus sanguinis effectively defends them against undesirable obstruction of growth [36]. The long term duration of HOSCN activity on the cells has the effect of irreversible inhibition as they are modified in to intermediate as R-S-OH and R-S-SCN products [35].

The concentration of iodides in the saliva is below 01μM;hence, their physiological oxidation by LPO is marginal as compared to thiocyanates. HOI, IO^-, as well as I_2, I_3^-, and I_2OH^- are the products of the reaction between I^- and salivary peroxidase Compound I [37, 38]. These products have a broader range of activity than hypothiocyanite, as they oxidize NAD (P) H and thioether groups in addition to the thiol moieties of proteins [25, 39]. In addition to the bacteriostatic effect of hypothiocyanite, the products of iodide oxidation show bactericidal and fungicidal activity. Van den Abbeele et al. observed a positive effect of mouth rinse on the reduction of dental plaque in vivo studies using a mouthwash containing OI^- [40]. Comparing the efficacy of antifungal activity on Candida blastopores, the survival rate in the LPO system containing thiocyanate comprised 55–88%, whereas it was just 0–4% in the system iodides applied [41]. Ariz et al. observed greater effectiveness of the iodide system in limiting the growth of C. Albicans blastoconidial biofilms on titanium surfaces in comparison to the LPO–H_2O_2–SCN^- system [42]. OI^- ions have a broad biocidal activity range, including fungi and Gram-negative and Gram-positive bacteria, and show synergy with SCN^- ions in the LPO system (stronger activity than SCN^- alone) [43]. Schlorke et al. presented another possible product of the LPO system—cyanogen iodide (ICN)—which is formed in the presence of both SCN^- and I^-. The strong toxic effects of this product are seen; however,the exact mechanism of its action on microorganisms is unknown. Although the physiological formation of ICN does not occur, it seems that such selection of the SCN^- and I^- ratio in dentifrices generates significant amounts of cyanogen iodide and may be important in increasing their antimicrobial efficacy.

Effects of the LPO system on bacterial cells&Dental Plaque:

The LPO system with the same composition is also been tested to assess its effects on the viability and lyase activities of bacterial cells. Approximately 107 CFU/ml of F. nucleatum

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or P. gingivalis was incubated with 30 ml of the LPO system in 40 mM citrate buffer (pH 5.0) at 37°C for 10 and 30 min under anaerobic conditions. Bacterial viability was assayed on TSA plates by spreading aliquots of serial 10-fold dilutions of the suspension. Bacterial colonies were counted after culturing for 6 days. The residual suspension was mixed with 300 µl of sodium azide is terminate the reaction of the LPO system. Bacteria were harvested by centrifugation at 3,000-× g for 15 min at 4°C, washed, and then re-suspended in 40 mM phosphate buffer (pH 7.7) with 50 mM sodium chloride to a turbidity of 1.5 at 550 nm. Lyase activities in these bacterial suspensions were measured in the same manner as described above. The influence of pH on the activities of the LPO system was also examined.

In both caries and periodontitis, the formation of plaque or biofilm is a key element in the path mechanism of these diseases. In the course of caries, the biofilm has supragingival localization [44] but is subgingival in periodontitis [45]. The LPO properties inhibit the formation of biofilm at each stage of its formation performed. Due to the ability of adsorption on salivary pellicle [46], the effectiveness of the LPO system has been proved in preventing the adhesion of precursor cariogenic microorganisms.

Clinical Application

Commercially available dentifrices containing lactoperoxidase have been subjected to clinical trials involving participants from various age groups with various clinical conditions, e.g., malodor, xerostomia, caries, and chronic periodontitis, as well as healthy subjects. The usefulness of LPO preparation was also determined during pilot studies in neonates under mechanical ventilation to avoid ventilator-associated pneumonia (VAP). In vitro studies on the effect of lactoperoxidase on oral microorganisms are being constantly published and document its effects against Streptococcus mutants in caries, when used separatelyor in combination with lactoferrin, lysozyme, or immunoglobulins [. The ability of S. mutans to create biofilms has been taken into account together with the activity of glucosyltransferases responsible for the synthesis of exopolysaccharides that build the biofilm matrix. It has been observed that the mouth rinse foam effectively reduces the retention of biofilms produced by salivary as well as non-pathogenic bacteria of the oral cavity. In addition to S. mutans, studies also concentrate on other oral streptococcispecies associated with the occurrence of periodontal diseaseand C. Albicans fungi.

LACTOFERRIN:

Lactoferrin on the lifestyle of oral microbiota:

The iron content in human saliva ranges from 0.1 to 1.0 µM depending on meals, but it might increase the gingival bleeding caused due to infection and inflammatory processes. During the infection and inflammatory processes, the recruitment of neutrophils increases saliva Lf concentration from 20 to 60 µg/ml (52).

Therefore, the saliva represents an interesting model to investigate the influence of iron and Lf concentrations on bacterial infections. The difference ratio between iron and Lf sometime plays an important role in the lifestyle of several bacteria (53, 54) by indulging aggregation and biofilmexpansion (52). Iron and Lf content, Apo-Lf (iron unsaturated form) was found to increase S. mutans aggregates and biofilm formation, whereas iron-saturated Lf were found to decrease aggregation and biofilm development in saliva pool (52). Similar behaviour was obtained in the periodontopathogen Aggregatibacter (Actinobacillus) actinomycetemcomitans: iron limitation up-regulates its biofilm genes contributing to biofilm formation (55).

The reported data suggest that to assess the effect of Lf in the oral cavity it is necessary to evaluate preliminarily the iron content of saliva. In fact, in periodontitis patients it is observed that the high iron concentration and the presence of hemin, might form complexes with Lf, together with Lf degradation by bacterial and human enzymes (56, 57), could be responsible, in vivo, for the lack or reduced activity of Lf even if its concentration increased following infection and inflammation.

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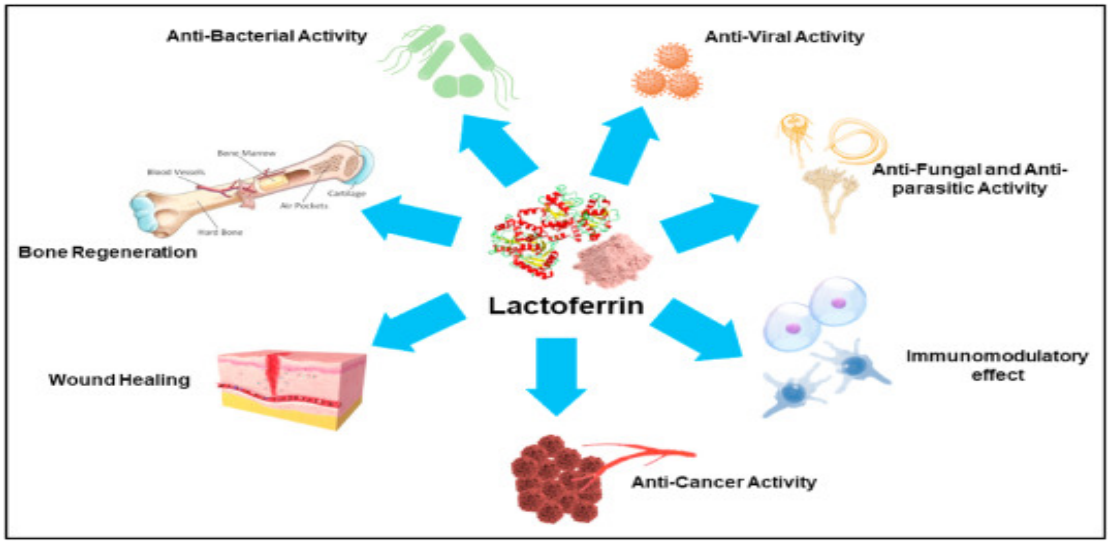

Inhibition of bacterial adhesion on abiotic and cell surfaces by lactoferrin

Abiotic surfaces:

Microbial adhesion and subsequent colonization, resulting in biofilm formation on abiotic surfaces such as dental surfaces and medical devices as dental prostheses, represents both a physiological process and a serious problem that can lead to oral illness. Efforts to control microbial adhesion by anti-adhesive new materials or compounds have had modest success once applied to the patient. Consequently, it might be helpful in discovering other compounds which are able to hinder microbial adhesion. The ability of Lf, in both Apo- and iron-saturated form, to inhibit the adhesion of S. mutans to hydroxyapatite (HA), mimicking tooth surface (47), may represent an interesting function. The demonstration observed that Lf inhibits the adhesion of S.mutans to a salivary film and HA through residues 473–538 of its C-lobe (48), which further helped to understand activity, which is unrelated to its iron-binding properties. Both Apo- and iron-saturated but also inhibit adhesion of free and aggregated S. mutant cells to a dental polymer. When Lfs is pre-coated to dental polymer or bound to both dental polymer and bacterial cells (52). Apo-Lf but not iron-saturated Lf also inhibits the attachment on HA of Prevotella nigrescens by binding to both HA and bacteria (49). Apo-Lf reduces the initial attachment of the commensal Streptococcus Gordonii by iron sequestration, but not that of periodontopathogen Fusobacterium nucleatum and P. gingivalis. Interestingly, the initial attachment of mixed populations of S. gordonii/F. nucleatum and S. gordonii/P. gingivalis is decreasedsignificantly in the presence of respect to that observed in the absence of Lf (50). In other studies, Lf is involved to inhibit the adhesion of A. actinomycetemcomitans and P. intermedia were found to be reconstituted basement membrane, by ionic binding, and P. intermedia to bacterial adhesions by specific binding of Lf (51).

The different nature of abiotic surfaces, the varying microbial adhesion mechanisms and the difference in vitro experimental conditions can explain the different results which are obtained for inhibition of bacterial adhesion by Apo- or iron-saturated Lf, which in some cases requires only ionic binding to biomaterials, and in others specific binding to bacterial structures.

Inhibition of microbial by Lactoferrin

Some mucosal pathogenic bacteria are capable of not only just adhering butare also capable of entering into non-professional phagocytes, such as epithelial cells. Inside host cells, bacteria obtain a protective niche by which they can replicate and persist, thus evading host defences. Virulence determinants, such as surface proteins are able to bind host cells which play a key role in approaching inside the host cells. Lf is involved to inhibit the entry of facultative intracellular bacteria, both Gram-negative and Gram-positive ([59]). Recently, the capability of S. mutans to enter inside gingival fibroblast cells has been reported [58]. Preliminary experiments show the anti-invasive activity of Lf against S. mutans infecting gingival fibroblasts.

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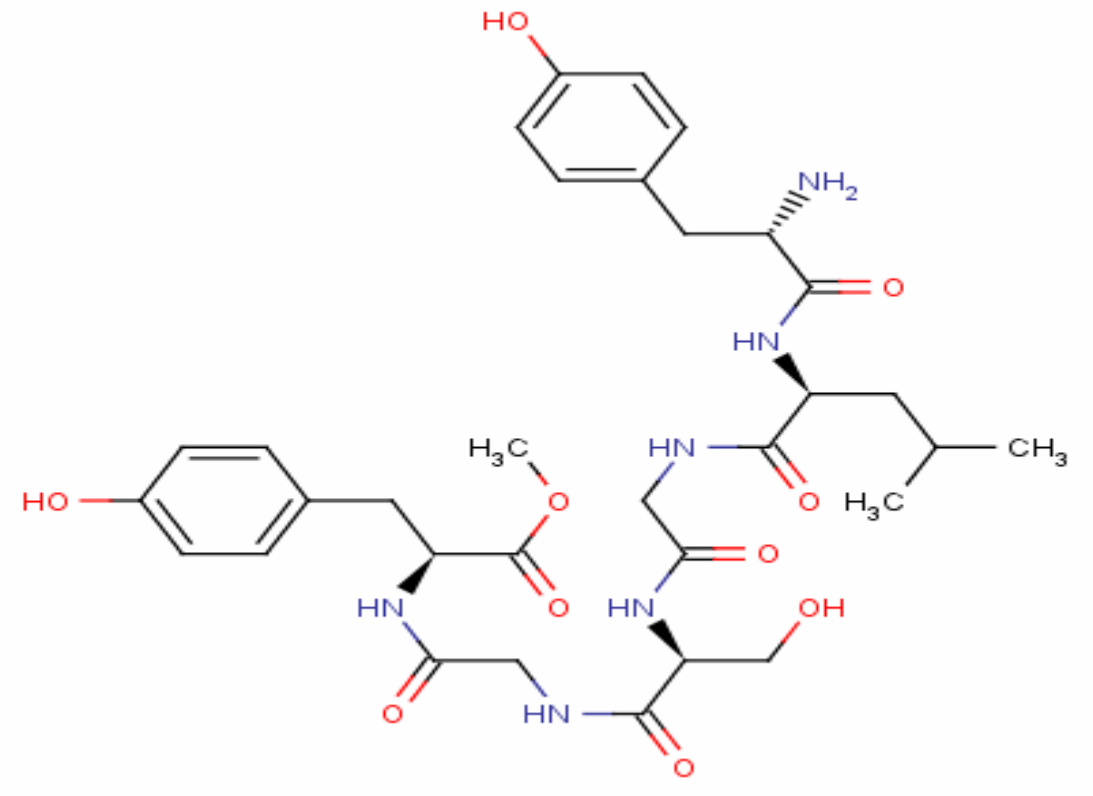
Structure of Lactoferrin:



LF AS PERIODONTAL INFLAMMATION MARKER

Components of CGF are used in identifying or diagnosing active disease and also to anticipate the risk of contracting it, along with to determination in its progression. The response of neutrophil granulocytes plays an important role in periodontal disease. CGF non-specific defense system can be determined through cytokines and neutrophil lysosomal enzymes, proteases like collagenases, or intracytoplasmic enzymes such as dehydrogenase lactate aspartate aminotransferase which allow monitoring progression of periodontal disease.[60]Recently, lactoferrin has been used as a biochemical marker owing to its ability to participate in several biological processes associated with periodontal disease. From 1993 onwards, studies have achieved to correlate lactoferrin in CGF as an effective marker for the number of polymorphonuclear neutrophils present in periodontal disease.[61]

Lactoferrin released by polymorphonuclear leukocytes into the CGF is a good indicator of periodontal inflammation since a strong relationship has been demonstrated among clinical parameters like CGF volume, depth when probing, levels of epithelial insertion, and plaque index. Lactoferrin levels in CGF (ng/site) increase up to 20 µM in the crevicular gingival fluid of patients affected with juvenile periodontitis, gingivitis, and adult periodontitis about inflammation severity, therefore, its quantification detects the degree of periodontal inflammation.[62] It has been shown there are differences in inflammatory response among periodontally healthy individuals during experimental gingivitis. In the search for markers that warrant the differentiation of inflammatory response between cases of gingivitis as compared to periodontitis, levels of elastase, (azurophilic granules) and lactoferrin (specific particles) in CGF of three different sites have been determined. The sites were the following: inflamed sites in gingivitis cases as well as inflamed sites in periodontitis cases, with and without tissue damage. It was found that elastase levels increase only in periodontitis cases, in contrast with lactoferrin which increases in both diseases, this suggests that patients afflicted with periodontitis experience a greater rate of cell release and the specific response of the host-associated to granulocytes.[63] Another study reports no significant differences in absolute values and

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elastase concentration, and a correlation of lactoferrin levels with activation of polymorphonuclear cells was achieved. It is important to note that in both studies it is suggested that the release of components of primary and secondary granules indicate alterations in the polymorphonuclear cells in different sites of both diseases.

In 2009, research has been conducted achieving an experimental gingivitis longitudinal study to assess the efficiency of lactoferrin as a possible marker of the disease progression. This study assesses lactoferrin levels in CGF and blood. The study showed that local inflammation created by the accumulation of dental plaque causes an increase of LF levels in the blood and CGF, and re-establishing oral hygiene decreases LF levels, although alterations are statistically insignificant.

Lactoferrin bacteriostatic and bactericidal activities which are present in saliva and crevicular gingival fluidsare considered as a part of mucosa defense mechanisms.When periodontitis afflicted sites were compared to healthy ones its levels increase.[64] Its concentration after surgical treatment has been assessed associating changes in stimulated and non-stimulated saliva. It has been found that LF is appropriate to monitor periodontal treatment results since high LF concentrations in the parotid gland and saliva in patients suffering aggressive periodontitis decrease in the CGF after surgical treatment in both salivas.

BIOFILM FORMATION INHIBITION

Biofilms (BF) are microbial communities that represent the most successful colonization configuration among microorganisms; they are ubiquitous and are responsible for many diseases. They are considered microorganism's communities and grow embedded in a self-produced exopolysaccharide matrix; they adhere to inert surfaces or live tissues. Development of biofilms occurs as a continuous process which includes the following development phases: a) conditioning, b) adhesion, c) extracellular matrix synthesis d) maturation, and e) dispersion. After the biofilm maturation, dispersion sets in, either of isolated cells or in conglomerates, which colonize new surfaces initiating thus a new cycle for the formation of biofilms. There are differences in the characteristics in detaching cells; since sessile (adhered) cells can keep the biofilm functionality (e.g. resistance to antibiotics), isolated cells, instead, can present a planktonic (free) phenotype and be susceptible to the hosts defehost'sechanisms.

Bacterial biofilm is the primary etiological factor for periodontal disease. Gram-negative and Gram-positive bacteria possess factors of virulence among which some are the following: lipopolysaccharide (LPS), fimbriae, lipoteichoic acids, peptidoglycans, formyl-methionine peptides, proteases, thermal shock proteins, and toxins among others which can cause direct or indirect damage on periodontal tissues, stimulating the host cells to activate the onset of inflammatory response causing gingivitis and in some cases periodontitis.[65]

Periodontal disease, of great incidence in humans, is characterized by an inflammatory process that results in loss of support for the tooth. The process begins with the formation of a glandular origin film (saliva, mucus) which covers mucosa and dental and epithelial surfaces of the gum. The first colonizers arrive shortly afterward and offer the means for the retention of other microorganisms, this allows for the formation of a diverse cellular community or biofilm. Uncontrolled development of resident microbes in these communities can contribute to the development of oral diseases.

Lactoferrin'sability to chelate ions as well as to interact with microbial components enables the modification of microorganisms' interactions with tissue surfaces. This is the reason why LF interference capacity in biofilms development phases has been explored. It has been reported that LF suppresses the initial union of S. Gordonii, as well as the coaggregation of this bacteria through iron abduction. This finding leads to the inhibition of the initial phase and development of the oral biofilm. It nevertheless has been shown there is no effect on P gingivalis or F nucleatum.

Moreover, LF prevents biofilm formation on bacteria who have escaped initial death. In the presence of subinhibitory concentrations of LF (20 µg/mL) P. aeruginosa bind and multiply but fail in the formation of microcolonies or biofilms differentiated structures. This is because LF stimulates a special type of bacterial locomotion, which puts distance between daughter cells from the site of parental division. Due to these reasons, microcolonies cannot be established; this then demonstrates interference in early phases of biofilm development[66] and allows a strategy to prevent antibiotic resistance, which is related to the formation of these structures.

P. gingival and P. intermedia reside as biofilms in subgingival plaque. Recent studies have

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determined that allocating bovine lactoferrin to patients afflicted with chronic periodontitis decreases the number of these bacteria in the plaque. For these reasons, the biofilm formation ability of several forms of lactoferrin has been explored: human and bovine Apo (iron-free) native and holo (iron saturated) as well as lactoferrin (LFcinB). Inhibition of biofilm formation activity has been found in low concentrations. It has also been found that use combined with antibiotics enhances the effect. For all the aforementioned reasons lactoferrin use is proposed in the prevention and treatment of periodontal diseases.

TREATMENT:

Due to its multiple functions, lactoferrin use has been proposed for therapy. It has been reported that the use of lactoferrin, xylitol, or the combination of both in in vitro studies on the structure of P. aeruginosa biofilms has resulted in a viability reduction > 2 logs when used in combination. This is due to the disintegration capacity in the biofilm structure affected by the xylitol, and to the bacterial permeabilization performed by lactoferrin. For these reasons, the combined use of both molecules is currently proposed for biofilm elimination treatment.

Because of lactoferrin antimicrobial activity, formulae have been developed using activated lactoferrin (ALF) [67] thatpreventsthe interaction of bacteria with tissue. These preparations will generate benefits for human health, among them oral health (plaque control, mouthwashes, denture cleansing, etc.) as well as wound protection care, food protection, and the avoidance of biofilm formation in catheters, which has caused many hospital-related diseases.

Role of the individual enzyme as a drug:

Serrate Serratiopeptidase is a proteolytic enzyme that is produced by the non-pathogenic enters bacteriumSerratia sp. In addition, this microbe was first isolated from the silkworm's intestine iopeptidaseto allow the dissolution of its cocoon. 1 It has been used for years for reducing inflammation and pain due to surgery, trauma, and other inflammatory conditions. Serratiopeptidase acts as an anti-inflammatory agent to pacify mild to moderate pain and inflammation. Common conditions associated with pain and inflammation include arthritis, trauma, surgical wounds, and fibromyalgia,aim many other diseases in general practice. Additionally, SP helps to reduce fluid retention in affected areas, which contributes to proper drainage and faster recovery. LOXs are key enzymes that catalyze the biosynthesis of SPMs and nonspecific inhibition of NSAIDs affects native inflammation resolution. New generation NSAIDs are effective as COXII specific but their clinical applications remain questionable and so researchers find more concern on enzyme-based agents and are seeking more specific drugs like anti-inflammatory agents like serratiopep and similar enzymes which indirectly assist resolution of inflammation without affecting the LOX-catalyzed SPMs production. The action of SP reported in the research works is their direct effect on the movement of immune cells. Enzyme regulates recruitment of PMNs and other lymphocytes at the site of inflammation and also SP reduces capillary permeability induced by histamine, bradykinin, and serotonin; and breaks down abnormal exudates and proteins thus, facilitating the atidasebsorption of decomposed products through blood and lymphatics. 2, 3 SP has also been shown to increase the clinical activity of many antibiotics including ampicillin, cephalexin, minocycline and it can be useful in dental infections since the activity of antibiotics will be enhanced along with the anti-inflammatory action of SP. In dentistry, it has also anti-inflammatory, anti-endemic, and analgesic effects; it is widely used after removal of wisdom teeth, postoperatively after maxillofacial surgeries, and in TMJ arthritis. In addition, SPwase found to improve trismus in a better way than corticosteroids. 4 Although, SP has antiemetic, analgesic, fibrinolytic, and chitinolytic properties its application in post-op oral surgeries or maxillofacial trauma has satisfactory results but due to its fibrinolytic activity the use of SP then dental abscess is controversial or we can say it is generally not recommended to prescribe the SP in dent alveolar abscess as it can further lead to the spread of the infection into deeper spaces and may lead to more complication. The reason for these complications is that hemorrhage, fibrin, and inflammatory cells surround an abscess, and around the necrosed part, fibrin is deposited in the form of a membrane and thus, it separates the dead part from the living. The wall of the abscess is formed by the effused and organized fibrin and sometimes the fibrin wall is not strong enough; in those cases, the pus finds its way into surrounding cellular texture and results in a diffuse abscess. 5 SP can be prescribed either as a single salt with a dosage of 5, 10, 15 mg BD or TDS or as an affixed-dose combination with other NSAIDs like PCM &

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 481-495 www.ijprajournal.com ISSN: 2249-7781

aceclofenac (325 + 100 +15 mg), diclofenac (50 + 10 mg), etc. The usual adult dose of SP ranges from 15 to 60 mg per day. The concomitant use of SP with aspirin should be avoided as anticoagulants interact with SP and might reduce its effects and the general consideration that should be taken to take this medicine is to take it with its food to avoid the GI upset. Like other drugs, Stooohase has few adverse effects that may include anorexia, GI upset, skin rashes, and epistaxis [68].

II. CONCLUSION:

From the overall discussion, it can be said that the use of enzyme therapy through the oral route has good results in the anti-inflammatory, analgesics roespeciallylly moreover with tissue healing has been observed in studies. In addition, we learn the uses of enzyme in various medicated product as well, as how they work, inhibition of bacteria, their use in drugs, etc.

Abbreviations:
LPO - Lactoperoxidase
GOx - Glucose oxidase
LF- Lactoferrin
SP = Serratiopeptidase,
LOX = lipo-oxygenase,
COX = cyclo-oxygenase,
TC = trypsin-chymotrypsin,
SPM = specialized pro-resolving mediators,
BR = bromelain,
NSAID = nonsteroidal anti-inflammatory drugs.
NHPM= National Health Promotion Movement

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