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In vitro studies of a degradable device for controlled-release of meloxicam

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Abstract

Background: Plaque biofilm and associated host responses are the primary factors in the pathogenesis of periodontitis. Delivery of medications directly into the periodontal pockets to suppress or eradicate the pathogenic microbiota or modulate the inflammatory response has attracted significant interest to limit periodontal tissue destruction. The aim of the present study was twofold: (1) to describe the development of a biodegradable controlled-release device containing meloxicam as the therapeutic agent and (2) to evaluate the in vitro release of meloxicam from this device into different release media.

Methods: Films of cross-linked gelatin matrix containing meloxicam were prepared, hardened for various time periods and cut in a form to fit to the periodontal pocket anatomy. The release of active agents was studied separately in 10 ml distilled water, artificial saliva and pH 7.4 phosphate buffer at 37°C. Apparatus Vibrax was used at 120 r.p.m. Determinations were carried out spectrophotometrically, and the release profiles were plotted as a function of time. The results were evaluated by the similarity test.

Results: The release rates of meloxicam from the hardened (1h, 4h, 8 h) formulations were slower than the unhardened formulation in all the three release media. Increasing the hardening time decreased the release rates. The overall release rates were similar in artificial saliva and pH 7.4 phosphate buffer, while it was lower in distilled water.

Conclusions: As a conclusion, cross-linked gelatin matrix films may be considered as a suitable inert material for obtaining a prolonged local release of meloxicam as an adjunct to the mechanical periodontal treatment. As required, further in vitro and in vivo studies will be performed before starting clinical applications of this controlled-release formulation of the anti-inflammatory agent.

Key words: controlled drug delivery; gelatin matrix; meloxicam; periodontal disease; rate measurements

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The eicosanoids comprise a group of inflammatory mediators derived principally from the metabolism of arachidonic acid that is present in mammalian cell membrane phospholipid pools. Currently, the local production of prostaglandins (PGs) and other arachidonic acid metabolites is widely accepted as a key factor in periodontal destruction (Offenbacher et al. 1984, 1986). One mechanism involved in periodontal tissue destruction is the prostaglandin E_2 (PGE_2)-mediated upregulation of tissue and bone-destructive proteinases, such as the collagenases (Wahl & Mergenhagen 1988, Wahl & Corcoran 1993). Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the production of eicosanoids primarily by blocking the cyclooxygenase (COX) enzyme systems that convert arachidonic acid to the individual metabolites. Recent research has shown that there are two related forms of the COX enzyme: COX-1 and COX-2. COX-1 is the predominant constitutive form of the enzyme and is expressed widely throughout the body. It generates PGS that protect the stomach and kidney from damage. COX-1 is also expressed constitutively in several tissues including the brain, reproductive organs, kidney and placenta during late gestation. However, COX-2 is usually absent in most tissues, but is rapidly expressed in response to a number of inflammatory stimuli, such as cytokines, and produces PGs that contribute to pain and swelling during inflammation (Vane & Botting 1998, Brooks et al. 1999, Warner et al. 1999).

The term ''host modulation therapy'' was first introduced over a decade ago

by Golub et al. (1992) to describe the emerging concept of managing the periodontal patient, in part, by the use of pharmaceutical or biologic agents to modify the patient's host response to bacterial irritants. Extensive data acquired in animal and human clinical trials have demonstrated the potential clinical utility of NSAIDs in periodontal treatment by reducing the inflammatory destruction. However, prolonged systemic administration of classical NSAIDs has limitations such as gastrointestinal upset, haemorrhage, and renal and hepatic impairment because of their adverse effects arising from the inhibition of COX-1. On the other hand, selective COX-2 inhibitors have very few side effects, if any, because of their preferential inhibition of COX-2 rather than COX-1. Most of the NSAIDs currently available are preferential COX-1 inhibitors while more recently developed NSAIDs are proclaimed as preferential or selective COX-2 inhibitors (Osiri & Moreland 1999). The selective COX-2 inhibitors are likely to exhibit a better clinical risk/benefit ratio than the classical NSAIDs. Meloxicam is a new NSAID of the acidic enolic class preferentially inhibiting COX-2 enzyme and is not only a potent inhibitor of acute exudation but also an intense inhibitor of bone and cartilage destruction (Engelhardt 1996). Meloxicam was reported to be effective in preventing alveolar bone loss in experimental periodontitis in rats with a better risk/benefit ratio than the non-selective COX inhibitors (Bezerra et al. 2000). Moreover, in our previous studies, we have evaluated the effects of adjunctive systemic administration of meloxicam on the clinical periodontal indices as well as on gingival crevicular fluid (GCF) levels of interleukin-4 (IL-4), IL-6, interferon-γ (IFN-γ), transforming growth factor-β 1 (TGF-β1) (Buduneli et al. 2001) and matrix metalloproteinase-8 (MMP-8) (Buduneli et al. 2002). From the results of our previous studies, it has been suggested that selective COX-2 inhibitors used as an adjunct to the non-surgical periodontal therapy may provide additional benefits by inhibiting GCF IL-6 and the pathologically elevated MMP-8 levels.

The topical administration of COX-2 inhibitors, on the other hand, is an alternative method to deliver these agents. Considering the local production of inflammatory mediators in the periodontal pocket environment and also the relatively inaccessible nature of the periodontal pocket for mechanical treatment, intra-pocket delivery systems are promising as adjunctive treatment modalities. Intra-pocket delivery systems may provide further advantages, possibly by reducing the total amount of the active agent used for treatment. Therefore, the aim of the present study was twofold: (1) to describe the development of a meloxicam containing controlled-release formulation in a gelatin biodegradable polymer matrix to be used as an adjunct to the conventional mechanical periodontal treatment and (2) from this described formulation, to evaluate the in vitro release profile of meloxicam in various release media.

Materials and Methods

Preparation of meloxicam-loaded films

The film was prepared by the solvent evaporation technique. One gram hydrolysed gelatin matrix was dissolved in 9 ml distilled water. Then, 0.3 g glycerin was added and 100 mg meloxicam was dispersed in this solution. The mixture was poured on teflon plates (5.5 × 17 cm) (Horoz Polyester Co., İzmir, Turkey) and evaporated at 37°C overnight. Gelatin matrix film containing 10% meloxicam was formed. The film was then cut into a desired form (∼ 2 mg; 0.6 × 0.7 × 0.1 mm width, height, thickness, respectively) with the aid of a cutting machine so that the cut pieces would fit to the periodontal pocket anatomy. The degree of the protein matrix degradation was altered by cross-linking reaction using formaldehyde. For this purpose, the cut films were hardened with 10% formaldehyde solution in isopropyl alcohol for 1, 4 and 8 h and then washed with acetone and dried at room temperature. Meloxicam was kindly provided by Nobel Drug Industry as a gift (İstanbul, Turkey). All other reagents used in the present study were of analytical grade.

Release studies

The release studies were carried out three times separately for each of the three different release media. Ten millilitres of distilled water, pH 7.4 phosphate buffer (USP XXII) and artificial saliva were used in each release study at 37°C at 120 r.p.m. For this purpose, apparatus Vibrax (IKA-VXR, Staufen, Germany) was used. Three millilitres aliquots of sample were assayed at predetermined time intervals (distilled water: 0, 1, 2, 3, 4, 5, 6, 24 h, pH 7.4 phosphate buffer: 0, 1, 2, 3, 4, 5, 6, 7, 24 h and artificial saliva: 0, 1, 2, 3, 4, 5, 6, 7 h) for meloxicam by measuring the absorption peak at 362 nm on a spectrophotometer (Shimadzu UV-1208, Kyoto, Japan). The release profiles were plotted as a function of time in terms of percentages. The final released amount of meloxicam at the end of the 24 h observation period was considered as 100%, and the released amounts on other sampling time points were compared with this 100%. The release results of meloxicam were expressed as the arithmetic mean values of the triplicate studies for each of the three release media. The Student *t*-test and the similarity tests were performed to compare all the release results with each other for each of the three different release media and also for each of the different hardening times. For the similarity test, the equation formula below was used, and a similarity factor ($f2$) greater than 50 was regarded as the sign of similarity.

$$f_2 = 50 \log \left[\frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} \left[\bar{R}(t) - \bar{T}(t) \right]^2}{n}}} \right]$$

(http://www.fda.gov/cder/guidance/1713bp1.pdf).

Results

Release results of meloxicam in distilled water

The release results in distilled water are given in Fig. 1. When the effects of hardening times on the release rate were compared by the similarity test, it was observed that the release rates of meloxicam from the hardened (1h, 4h, 8 h) formulations were slower than the unhardened formulation, and, according to the similarity test, their release profiles were not similar to the release profile of unhardened formulation ($f_2 < 50$, $p > 0.05$). The effect of hardening times of 4h and 8 h on the release rate was found to be similar ($f_2 > 50$, $p < 0.05$) and they prolonged the release of meloxicam more effectively than 1 h hardening, which was not similar to these results ($f_2 < 50$, $p > 0.05$).

Release results of meloxicam in artificial saliva

The release results in artificial saliva are given in Fig. 2. The unhardened formulation released meloxicam faster than the hardened formulations. The similarity test revealed that the release profiles of hardened formulations (1h, 4h, 8 h) were similar ($f_2 > 50$, $p < 0.05$).

Release results of meloxicam in pH 7.4 phosphate buffer

The release profiles of all formulations in pH 7.4 phosphate buffer are shown in Fig. 3. The similarity test showed that the release profiles of hardened formulations (1h, 4h, 8 h) were similar to each other ($f_2 > 50$, $p < 0.05$). The release of meloxicam from the unhardened formulation was more rapid than the hardened formulations ($f_2 < 50$, $p > 0.05$).

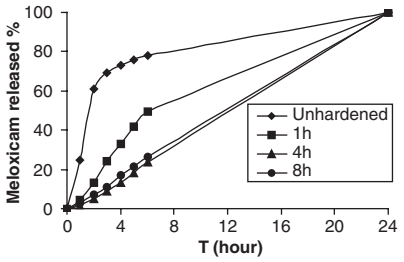

Fig. 1. Release results of meloxicam in distilled water.

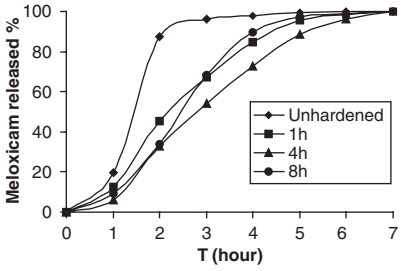

Fig. 2. Release results of meloxicam in artificial saliva.

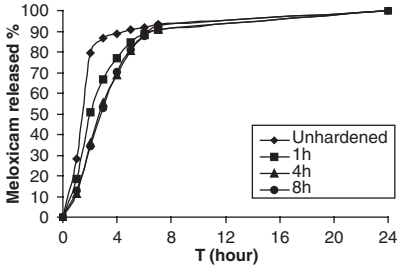

Fig. 3. Release results of meloxicam in pH 7.4 phosphate buffer.

The effects of release media on the release rate of meloxicam were also evaluated. All the release profiles (unhardened, 1h, 4h and 8 h hardened) observed in artificial saliva and pH 7.4 phosphate buffer were found to be similar according to the similarity test. The release of meloxicam in distilled water was slower than the other two release media.

The release profiles of all formulations were in accordance with the first-order kinetic model, and their release rates were calculated (Table 1). The fastest release rates were obtained from the unhardened formulations. The release rates were decreased by increasing the hardening time. The lowest release rate was observed in distilled water because of the low solubility of meloxicam.

Discussion

A local drug delivery device consists of a drug reservoir and a limiting element that controls the rate of drug release. This study was performed to develop a controlled-release formulation of meloxicam to be used in the periodontal pocket as an adjunct to the conventional mechanical periodontal treatment.

The main benefits of a controlled-delivery device include improved patient compliance, improved pharmacokinetics, improved drug access to the site of disease and lower total drug dosage (Goodson 1989). Moreover, systemic administration has drawbacks like the side effects, particularly on the gastrointestinal system. Furthermore, the physical characteristics and working properties of the delivery system may influence the degree of acceptance by the professional community as well as the patient population. The present study indicates that a selective COX-2 inhibitor, meloxicam, shows a sustained rate of drug release from gelatin matrix over extended periods of time.

Two isoforms of COX catalyse the synthesis of PGs from arachidonic acid: COX-1 and COX-2. PGE_2 not only induces vasodilatation and capillary permeability but also stimulates osteoclastic bone resorption (Rifkin et al. 1980). Elevated levels of PGE_2 have been detected in gingivae and GCF of patients with chronic periodontitis (Offenbacher et al. 1986). Selective COX-2 inhibitors are likely to exhibit a better clinical risk/benefit ratio than the classical NSAIDs (Engelhardt 1996). Selective COX-2 inhibitors have attracted great interest in the field of periodontal treatment because of their good effectiveness as well as good tolerance. Adjunctive use of either systemic or local NSAIDs provides additional reductions in both gingival inflammation and alveolar bone resorption (Jeffcoat et al. 1988, Reddy et al. 1993). However, the side effects of the non-selective NSAIDs preclude their routine and long-term administration in periodontal treatment. Many of the side effects limit the usage of non-selective NSAIDs. COX-1 plays a role in platelet aggregation, haemostasis and the protection of gastric mucosa. COX-2 is a crucial mediator of pain, inflammation and fever. Non-selective NSAIDs inhibit PGs, broadly inhibiting both COX-2 and COX-1 isoenzymes. NSAIDs' toxic effects are mostly caused by COX-1 inhibition in the stomach, kidney and platelets. COX-2 inhibitors were developed for the treatment of inflammation while avoiding adverse reactions caused by COX-1 inhibition, especially in the gastrointestinal system (Osiri & Moreland 1999). These agents offer potentially significant advantages because of their relative lack of gastrointestinal tract irritation. Because of this, these medications are likely to be frequently used in the management of dental and medical conditions (May et al. 2001). Recently, we have used systemic meloxicam as an adjunct to scaling and root planing in the treatment of chronic periodontitis patients and observed promising inhibitory effects of meloxicam on the GCF levels of IL-6 (Buduneli

Table 1. Release rates (h^{-1}) of meloxicam in distilled water, artificial saliva and pH 7.4 phosphate buffer

Hardening time	pH 7.4 Phosphate buffer	Artificial saliva	Distilled water
No hardening	0.337	1.239	0.221
1 h	0.369	1.051	0.129
4 h	0.396	0.628	0.050
8 h	0.423	0.522	0.045

et al. 2001) as well as MMP-8 (Buduneli et al. 2002).

COX-2 can be considered as a rate-limiting enzyme in the prostaglandin biosynthetic pathway. The continued expression of COX-2 over time could contribute to a subclinical inflammatory state leading to progressive attachment loss (Zhang et al. 2003). Oral rinse formulations of NSAIDs have been developed to be used in periodontal treatment in an attempt to avoid the gastrointestinal side effects associated with systemic use of non-selective NSAIDs (Kelm et al. 1996). However, oral rinse formulations are far from providing prolonged effects and only a controlled-release local delivery formulation can sustain the active anti-inflammatory agent in the periodontal pocket, while overcoming the systemic side effects. On the other hand, it is reasonable to think that different species of periodontal pathogens will stimulate COX-2 expression to varying degrees. Therefore, a controlled-release local delivery system of an anti-inflammatory agent either administrated alone or in combination with an antimicrobial agent could play a valuable role in periodontal therapy.

Recently, our group had conducted in vitro studies on development of cellulose acetate films containing chlorhexidine, indomethacin and meloxicam suitable for use in the periodontal pocket (Çetin et al. 2004). Among these two NSAI agents, the release pattern of the meloxicam-containing formulation was found to be much better than that of the indomethacin-containing formulation. The cellulose acetate formulation used in our previous study had released only 30% of meloxicam within 120 h, while the 8 h hardened gelatin formulation used in the present study released 100% of drug within 7 h. The release results in both studies were in accordance with the first order kinetic model (Notari 1971). When these two meloxicam-containing formulations are compared (cellulose acetate versus. gelatin films), it is quite clear that the release of meloxicam from the cellulose acetate films was much more promising than the release from the gelatin matrix. However, in vivo release profile, possibly modulated by the flow rate of GCF as well as its composition and the subgingival biofilm, may well differ widely from the in vitro release profile. Fluctuations in GCF content of substances like antibodies, cytokines and enzymes derived from host cells or oral bacteria may affect the release pattern of an active drug placed directly in the periodontal pocket within a degradable matrix.

Gelatin, the chief protein component in the skin, bones and white connective tissues of the animal body, has both clinical and pharmaceutical uses. Gelatin is practically insoluble in acetone, chloroform, ethanol, ether and methanol and soluble in water (Wade & Weller 1994, Keenan 1997). Gelatin granules used in the preparation of pastes, pastilles, suppositories, tablets, and hard and soft gelatin capsule shells dissolve rapidly in water over 35°C. The cross-linking of gelatin matrix by chemical means (formaldehyde, glutaraldehyde) is used extensively, and this so-called hardening permanently reduces the solubility of gelatin. This type of gelatin is used for controlled release of active substances. Dry gelatin stored in airtight containers at room temperature is known to have a shelf-life of many years (Wade & Weller 1994, Keenan 1997). Considering its well-documented properties suitable for use in an intrapocket drug delivery device, we have preferred to use gelatin as the degradable matrix in the present formulation.

On the other hand, when compared with the non-degradable cellulose acetate polymer, the biodegradability of the gelatin used in the present study may have an effect on the faster release rate of meloxicam. But the degradable devices have the great advantage of minimizing the patient visits, and also, they ensure patient compliance. Therefore, development of biodegradable intrapocket drug delivery devices has been the challenge. Better judgement on the suitability of the delivery system can be made only after conducting in vivo release studies in animal models using these meloxicam delivery systems. Our studies are only in the elementary stage and these formulations may be improved by further laboratory work. Thus, the higher meloxicam content (50%) in our previous formulation with cellulose acetate may also have had a role in the longer period of drug release compared with the present gelatin matrix formulation with less meloxicam content (10%). At present, we are working on different formulations including different matrix polymers and different rates of drug content to improve the release profile of meloxicam.

Local production of PGs is one of many likely mediators of periodontal tissue destruction, and human periodontal tissue COX sensitivity to the NSAIDs is currently being investigated. It is possible that specific NSAIDs are particularly good inhibitors of gingival tissue COX, whereas others are excellent inhibitors of the COX of alveolar bone cells (Howell & Williams 1993). Further studies are required to select or develop an optimal NSAID to be used in a local delivery system as an adjunct to conventional periodontal therapy. The optimal dosage of the active agent is another critical issue that needs to be determined by further studies.

In conclusion, it was observed that cross-linked gelatin matrix is a convenient inert material for obtaining a prolonged drug release. The hardening time has an effect on the release rates, and also the release media are important. Meloxicam is a selective COX-2 inhibitor and as yet, there exists no local delivery product of meloxicam in the market. COX-2 represents a valid pharmacologic target for the treatment and prevention of periodontitis and therefore, further in vitro and in vivo studies will be performed before starting clinical application of this formulation. If and when successful controlled-release formulations of anti-inflammatory agents are developed, the inflammatory cytokines and enzymes derived from the host cells can be effectively controlled, eventually preventing or slowing periodontal tissue destruction.

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