EFFECT OF MORPHINE DEPENDENCY ON BONE REPAIR PROCESS IN THE CORTICAL BONE OF TIBIA IN RATS

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Abstract- Several environmental factors have been found to affect the process of bone healing including malnutrition and smoking. To our knowledge, the effect of addiction on bone repair process has not been studied yet. This study was designed to investigate the effects of morphine dependency on bone repair process in rats. Fifty six rats were divided into two groups randomly, each group containing 28 rats. The rats in study group got morphine by adding morphine powder to their water for 21 days. Then morphine dependency was confirmed by injecting naloxon intra-peritoneally. Both groups underwent surgery and a fixed round hole was created in anteromedial aspect of the tibia. Bone biopsy was performed on days 3, 6, 10 and 20, on 7 randomly selected rats from each group and the results of microscopic study were analyzed by Mann Whitney test. There was statistically significant difference between the two groups according to neutrophilic exudates percentage and granulation tissue formation on 3rd day ($P < 0.005$), neutrophilic exudates percentage ($P = 0.01$) and immature bone formation on 6th day ($P < 0.005$), immature bone formation ($P = 0.001$) and mature bone formation ($P < 0.05$) on 10th day and mesenchymal tissue formation on 20th day ($P < 0.05$). It is concluded that the process of bone repair in a hole created in the rat tibia is markedly delayed in morphine-dependent rats.

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Key words: Morphine dependency, bone repair, rats

INTRODUCTION

Fracture healing is a well timed sequence of biological events leading to reformation of bone continuity. The process begins with the formation of a hematoma and continues through inflammatory stage, callus formation and finally remodeling stage (1). There are several well-known variables that influence fracture healing. They can be classified as injury variables, patient variables, tissue variables and treatment variables. Age, nutrition, systemic hormone and nicotine are the most important patient variables. It has been shown that malnutrition, cigarette smoking and alcohol consumption impair fracture repair process (2-5).

Morphine is one of the major alkaloids of opium. The chronic abuse of opioid drugs may be associated with accelerated bone turnover and reduced trabecular bone mass. One explanation of this bone turnover in heroin addicts may be the influence of hypopituitary-hypothalamic-gonadal axis (6, 7). The activity of both exogenous opiates and endogenous opioids are mediated through specific receptors that have been classified into three main groups, termed mu, delta, and kappa (8). Several studies have described the presence of opioid receptors in osteoblasts (9, 10). Reduced osteocalcin production by osteoblasts is considered to be a marker of low osteoblastic activity. Endogenous opioids may be involved in the reduction of osteocalcin observed in

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stressful situations associated with tissue injury (11). Serum levels of osteocalcin have been reported as being low in heroin abusers (12).

These studies suggest that opioid dependency may have an influence on bone repair process. We decided to study the effect of morphine dependency on bone repair process in rats.

MATERIALS AND METHODS

This study was designed as an experimental double blind study. Before starting the study the days for biopsy were assigned in a pilot study. Surgery was performed on 10 healthy male rats weighing 250-300 g.

Rats were anesthetized with ether. A longitudinal 2 cm skin incision was made through skin and fascia of the muscles at the level of tibia on its proximal and medial surface, about 10 -15 mm distal to the knee joint. A hole was then pierced in the cortical bone of tibia in the center of its medial aspect about 10 mm distal to the knee joint (diaphyseal region) using a 1.5 mm drill bit. The hole was made in such a manner as to penetrate the cortical bone and damage the trabeculae in the medullary canal underneath the hole but not to damage the contralateral cortical bone. Care was taken not to injure periosteum in the vicinity of the hole. In the above model there is spontaneous partial healing of the gap injury (probably due to its low diameter relative to the tibia width) at about three weeks after injury and a complete healing at a longer interval.

Biopsy was performed on several days in different rats and finally days 3, 6, 10 and 20 which were concordant with main pathologic changes and were determined as the appropriate intervals for collecting the newly formed tissue in the hole-injury.

A total of 56 rats were randomly divided into 2 groups and kept in the same environment. One group became dependent by giving a gradually increasing oral dosage of morphine as below. The initial dosage of 0.1 mg/ml in water was given in the first day. Additional doses of 0.2, 0.3 and 0.4 mg/ml were also given every 48 hours up to a maximum of 0.4 mg/ml which were continued to day 21 in the same period. The control group rats were given water. At the end of third week 10 percent of the population in each group (3 rats from each group) was randomly chosen and 2 mg/kg naloxon was injected. The rats were observed for 20 minutes in a glass container for withdrawal symptoms and the results recorded. Withdrawal symptoms in addicted rats were jumping, head tremor, diarrhea, irritability, claw tremor, eyelid drop, body's stretching and shivering. Rats were supposed to be dependent if four signs or more were present (13, 14). After confirming dependency to drug, surgery was performed in all rats.

On days 3, 6, 10 and 20 the newly formed tissue in the hole-injury was collected after 7 rats were scarified with an overdose of chloroform in each group. The specimens were held in formalin solution 10% for pathologic studies.

The results were analyzed for statistical difference among the groups using Mann-Whitney test. The results would be considered significant if the level of probability was 0.05 or less.

RESULTS

The histological study of the specimens taken at the 3rd day showed neutrophilic exudates in both control and morphine-dependent rats (neutophils were gathered in a fibrin background derived from plasma). In the morphine-dependent rats little granulation tissue formation (special tissue due to fibroblast and endothelial cells proliferation) was observed whereas in the control rats the amount of granulation tissue formation were significantly ($P < 0.05$) higher compared with the control (Fig. 1).

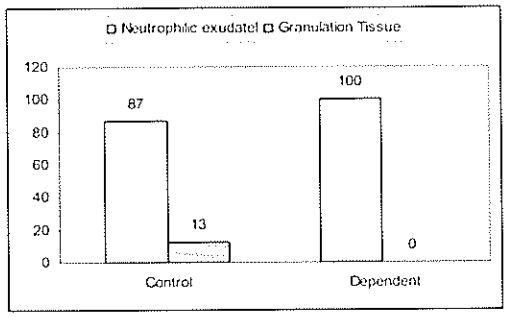

Fig. 1. 3rd day. Significantly higher granulation tissue in control group.

In the study at 6th day, granulation tissue was most prominent in specimens, but there was the presence of immature bone (the first bone tissue) during bone repair was considered as a clue of progression of fracture repair. We could see that the tibia of morphine-dependent rats presented significantly ($P < 0.005$) little immature bone formation (Fig. 2).

The study of specimens taken on tenth day showed that there was a significant difference in the amount of mesenchymal tissue ($P < 0.005$), immature bone ($P < 0.001$) and mature bone ($P < 0.05$) between study and control groups (Fig. 3).

The only significant result of the histologic examination at 20th day was the difference in amount of mesenchymal tissue between two groups ($P < 0.05$). The difference in amount of other tissues (cartilage, immature bone and mature bone) were non-significant (Fig. 4).

DISCUSSION

This animal experiment showed that morphine dependency has a significant inhibitory influence on fracture healing. The mechanism by which opioids can affect bone repair process remains unclear. Opiate-like activity of both exogenous opiates and endogenous opioids is mediated through interaction with specific cell surface receptors. It has been shown that osteoblast-like MG-63 cells express the three types of opioid receptors and opiates can reduce the activity of these cells presented as reduced osteocalcin activity (11).

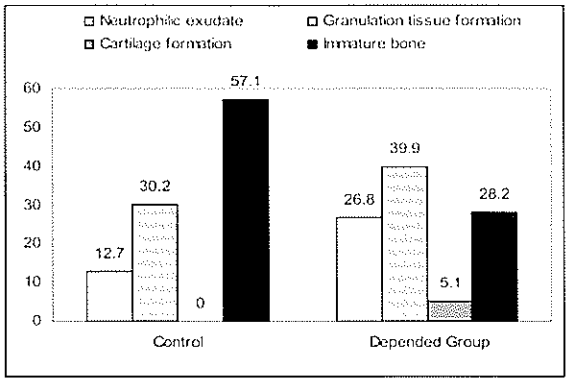

Fig. 2. 6th day. Immature bone (the first bone tissue) formation was significantly less in specimens of morphine-dependent rats.

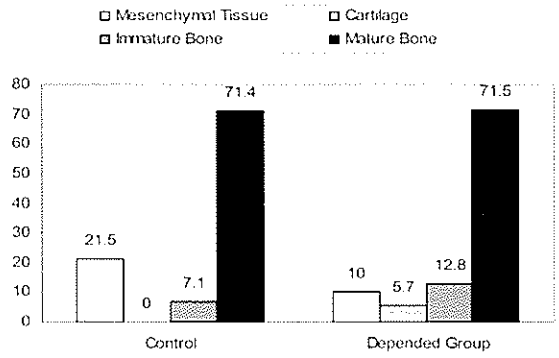

Fig. 3. Tenth day. High percentage of mature bone in control group compared with the study group.

Animal studies have shown that mobility, agility and activity of morphine dependent rats are less than the control rats. As a result, the extremity was exposed to stress and loading, and facture healing was not stimulated (15). This may explain why the healing process of morphine dependent group is worse than control group.

Opioids can stimulate HPA axis through activities of central opioid receptors. The activation of the HPA elicits the production of ACTH from pituitary that in turn elicits the release of glucocorticoids that could eventually affect bone healing process (15). It has been shown that prolonged systemic administration of glucocorticoids impaired fracture healing in the rabbits. The mechanism is not clear, but corticosteroid administration results in decreased osteoblast activity and it also adversely affects the metabolism of vitamin D (16).

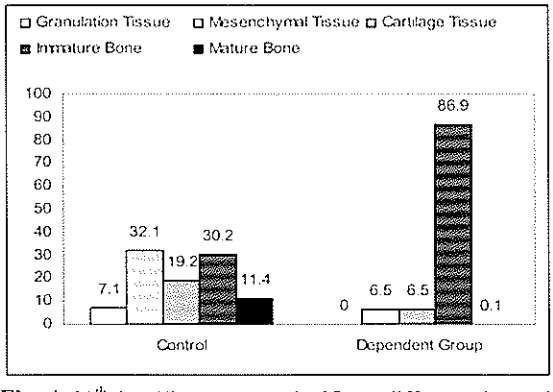

Fig. 4. 20th day. There was no significant difference in repair tissue of two the groups. Significantly higher mesenchymal tissue was observed in the control group.

In addition as we know, glucocorticoids have strong anti-inflammatory effect and in the literature, an inhibitory effect of NSAIDs on bone generation has been shown. From clinical experiences, it has been known for some time that the administration of NSAIDs has an inhibitory effect on the formation of heterotopic bone formation (17). It has also been shown in animal experiences that fracture healing is markedly delayed by the oral administration of diclofenac (18). The same result has been shown when indomethacin was used in a study. Fracture healing under unstable condition is characterized by callus formation and occurs in different phases. On the first day after the fracture an inflammatory response predominates. Prostaglandins are supposed to have an important role in this phase. The negative effect of indomethacin and diclofenac on bone healing has been described to their inhibitory effects on the production of prostaglandins (18). Several other mechanisms have been described for inhibitory effect of NSAIDs on fracture healing. They may influence bone metabolism via cytokine network, growth factors, free oxygen radicals and lysosomal enzymes. It has also been proposed that impairment of fracture healing by them may be caused by their analgesic effects, but this theory has not supported by several other studies.

With our results, we were able to show in this animal experiment involving rats that fracture repair is markedly delayed in morphine dependent rats.

Opiates are best known for their analgesic effect and their potential for abuse. Patient's overall health condition (malnutrition, smoking and corticosteroid consumption) should also be considered. Morphine and opium abuse in our community is notable and with respect to the higher complications of bone fractures in this specific group of patient, we decided to study the effect of this drug on the quality and quantity of bone union.

As described, progression of bone repair was significantly more rapid in control rats compared to morphine-dependent rats on 3rd, 6th and 10th days,. However on 20th day, it seems that bone repair progression was similar in both groups. This can be described to inappropriate late time of biopsy. Moreover, it was noted that, there was a significant difference in mature fibrous tissue among mesenchymal tissue in control rats on 20th day. It seems that significant inflammatory reaction in control groups was secondary to passing nylon strand from injured area and again this finding was confirmed in several other studies. It is obvious that other studies should be designed to confirm the above results and determine the precise mechanism of morphine effects on bone repair process.

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COMPARISON OF EROSION AND PERIODONTAL INDICES IN PATIENTS WITH AND WITHOUT GASTROESOPHAGEAL REFLUX DISEASE

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Abstract- Gastroesphageal reflux disease (GERD) is a systemic disease with intraoral manifestations. The aim of this study was to compare erosion (Loss of tooth structure due to a chemical process without bacterial cause) and periodontal indices including: calculus index (CI), plaque index (PI), gingival index (GI), clinical attachment level (CAL) and probing pocket depth (PPD) in patients with GERD and in non GERD subjects that was done in 2002 in Imam Khomeini Hospital. 35 Patients with GERD (test group) and 35 subjects without GERD (Control group) were selected randomly for this study. Statistical analysis for comparing differences between the test and Control groups were Performed using chi square and Fisher exact test. The results showed that the prevalence of erosion was significantly higher in test group (14.3% GERD, 62.9% non GERD). There was also a significant difference in GI, PI, CI and PPD between test and control groups. CAL did not show any significant difference between the two groups. Also *Helicobacter pylori* was significantly higher in test group (80% test, 54% control group). According to the results, communication between dentist and internist leads to diagnosis and control of GERD, and prevents changes of teeth and periodontal structures.

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Key words: Erosion, periodontal indices, gastroesophageal reflux disease

INTRODUCTION

The term "reflux esophagitis" was first described in 1946 to explain the reflux of irritant fluids from stomach to esophagus. The term gastroesphageal reflux disease (GERD) used to describe persons with clinical signs or histopathologic changes due to repeated course of GERD (1).

Relation between gastrointestinal disorders and erosion of teeth was reported for the first time by Bargon and Austin in 1973 in a patient with chronic vomiting. Reflux and acid mainly affect the palatal aspect of maxillary incisors but repeated reflux can lead to involvement of other teeth (2). Prevalence of erosion [loss of tooth structure during a chemical process without bacterial intervention (3)] has been reported to be from 5 to 42% in healthy populations but prevalence of erosion in patients with bulimia is higher (69%) (4, 5). All of the studies showed erosion in maxillary anterior teeth. There is not any report about soft tissue changes related to GERD (6). Only one study reported significant gingival changes in patients with GERD (7). The purpose of the present study is to compare the erosion and periodontal indices between patients with and without GERD.

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MATERIALS AND METHODS

A total of 70 subjects who attended the Gastroenterology Department of Imam Khomeini Hospital were included in this study. According to the internist's diagnosis based on patients' signs and symptoms, clinical examination and endoscopy, 35 subjects with GERD (test group) and 35 subjects without GERD (control group) were selected. Factors such as age, sex, tooth brushing and the frequency of eating sour foods were matched in test and control groups.

Exclusion criteria for selecting subjects in this study were: pregnancy, systemic diseases (epilepsy, anemia, diabetes mellitus, hepatitis), intake of alcohol, smoking and using drugs such as antibiotics and corticosteroids that could interfere with the condition of periodontal tissues. All of subjects were examined for *Helicobacter pylori* during endoscopy and all of the subjects must have had a minimum of 8 teeth in mouth. Presence or absence of erosion and the plaque index [(PI) (Silness and loe)], gingival index [(GI) (Silness and loe)], calculus index [(CI) (Ramfjord)], probing pocket depth (PPD) and clinical attachment level (CAL) in first molars and incisors of upper and lower jaw were measured by means of mouth mirror and William's periodontal probe (8). In the absence of above mentioned teeth, neighboring tooth was examined.

Statistical analysis for comparing differences between the test and control groups was performed using Chi square and Fisher's exact test.

RESULTS

In present study prevalence of erosion in test group was higher (62.9%) than the control group (14.3%) (Table 1). There was a statistically significant difference in CI, PI, GI and PPD between test and control groups, but CAL showed no difference between the two groups (Table 2).

Table 1. Prevalence of erosion in test and control groups*

Erosion	Case	Control	Total
No	13 (37.1)	30 (85.7)	43 (61.4)
Yes	22 (62.9)	5 (14.3)	27 (38.6)
Total	35 (100)	35 (100)	70 (100)

*Significant was obtained from χ^2 and Fisher's exact test $P = 0.000$.

Table 2. Periodontal indices in test and control groups

Index	Control	Test	*P* value
GI	0.5067±0.2657	0.7485±0.8600	0.000*
PI	0.4938±0.7900	0.6380±1.5057	0.000*
CI	0.6427±0.3057	0.8539±1.1271	0.000*
CAL	0.3138±2.0514	0.5397±2.1629	0.295
PPD	0.5488±1.9629	0.4785±2.2571	0.000*

Abbreviations: GI, gingival index; PI, plaque index; CI, calculus index; CAL, clinical attachment level; PPD, probing pocket depth.
* Significant.

Evaluation of *H. pylori* in test and control groups revealed higher prevalence of this organism in the stomach of test group (80%) in comparison to control group (54%) (Table 3).

DISCUSSION

Prevalence of GERD in developed countries is much more than what had been believed in the past. It is estimated that near 70% of adults suffer from GERD every day and more than 30% suffer from GERD every now and then.

All of the patients do not have any sign but sometimes erosion may be the first sign of GRED (9). Palatal aspect of maxillary teeth has been most severely affected area in all of the studies (4) but evaluation of oral mucosa has revealed only non specific symptoms such as sensitivity of tongue or other oral mucosa, burning sensation of mouth and oral ulcers (10).

Only one study reported periodontal findings. In that study, calculus was greater in healthy individuals but the difference was not statistically significant. The author suggested that lower calculus in GRED subjects is related to better oral hygiene in GRED group due to foul and acidic taste of mouth in relation to reflux (7).

Table 3. Prevalence of *H. Pylori* in test and control group*

H. Pylori	Control	Test	Total
No	19 (46)	7 (20)	26 (37)
Yes	16 (54)	28 (80)	44 (63)
Total	35 (100)	35 (100)	70 (100)

*Significant was obtained from χ^2 test (*P* value=0.003).

One of the major findings of our study is that the test and control groups were matched for tooth brushing, age, sex and frequency of taking sour foods, but PI, CI, GI and PPD were significantly higher in test (GRED) group.

CAL showed no significant difference between test and control groups. Higher prevalence of erosion in test group is in accordance with previous studies (1, 4, 6, 8). Another important finding of our study is higher prevalence of *H. pylori* in test group. According to possible relation between periodontal infection and *H. pylori* (11) and higher periodontal indices in our study it is possible that recurrence of reflux to the oral cavity that contains *H. pylori* leads to reaction with oral flora and in the presence of local factors such as plaque acts as a predisposing factor for periodontal disease.

To confirm the relationship between GRED and periodontal changes additional long term studies with greater number of subjects need to be performed to verify the real impact of GRED in the initiation and development of periodontal changes. Direct communication between dentist and internist is very important for diagnosis and treatment of GERD and periodontal changes.

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