

Original Article

Evaluation of wound healing and post-operative intra-abdominal adhesions in opium addicted rats

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Abstract

Introduction: Opium addiction can change immune response to Types of stress such as injury or trauma due to alterations in the in secretion status of cytokines in the body. In this study, effects of opium addiction on wound healing and post-operative adhesion bands were assessed after laparotomy.

Materials and Methods: Male rats (n=20) were randomly divided into opium addicted (documented with Naloxone test) and control group. Three weeks after surgery, site of abdominal incision was excised elliptically and sent for wound healing grading assessment by pathologist and an intra-abdominal adhesion band assessment was done. The concentrations of three cytokines (TNF α , IFN γ and IL10) were also measured before, immediately after surgery and 24 hour after surgery.

Results: Post-operative intra-abdominal adhesion was decreased in opium addicted group in comparison to control group (p value = 0.014). No statistically significant difference was found in the wound healing phase in two groups (P value = 0.057). Our findings showed that serum level of TNF α , IFN γ and IL10 in two groups measured in all phases of examination (before surgery, within 30-60 min after surgery and 24h after surgery), were not statistically different/significant (p>0.05).

Conclusion: Since opium addiction can decrease post-operative intra-abdominal adhesions in rats, they may be susceptible to increased inflammation and these effects may be due to decreased post-operative pain.

Keywords:

Opium Addiction;
Laparotomy;
Wound Healing;
Adhesion;
Rats

Received:

23 Aug 2015

Accepted:

15 Nov 2015

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Introduction

Opium addiction is a social problem in Iran, thus the number of addicted patients/population who has undergone surgical procedure has been on the rise

(Mansourian et al., 2012; Nemati et al., 2010). Opium addicts suffer a higher degree of post-operative morbidity as compared with non-addicts. A thorough and comparative analysis revealed that opium addicts had a significantly higher incidence of postoperative respiratory, cardiovascular, systemic and local

complications (Malviya et al., 2011). Also, opium addiction experience changes in their endocrine hormones (Cassidy et al., 2010; Lovallo, 2006) and cytokines (Nabati et al., 2013; Sido et al., 2004; Wadji et al., 2005) which may affect wound healing after surgery.

Wound healing is a complex process that involves interactions among a large number of distinct cell populations whose regulation is tightly controlled (Efron and Moldawer, 2004; Labat et al., 2001). Often, Wound care is complex, frequently time-consuming, sometimes confusing and almost always expensive (Alizadeh et al., 2011). Normal wound healing is the result of successful interactions occurring in the epidermis, the dermis, at the cellular level and the extracellular matrix, as well as a systemic response, including the cellular immunity, the humoral immunity, and the coagulation cascade (Efron and Moldawer, 2004). With cytokines being crucial to physiologic and pathologic process of wound healing, They have become a common target for modifying the repair response (Khaksari et al., 2012). Dysregulation in cytokine or growth factor expression dramatically alters the normal wound healing process (Abbasizade et al., 2014; Efron and Moldawer, 2004; Järvinen and Laiho, 2012). Successful wound healing is a complex process that requires coordination/interaction of multiple growth factors, cell types, and extracellular cellular matrix components. IL-10 has been demonstrated to be critical in the fetus' intrinsic ability to heal without scars, and, further, can induce scarless healing in post-natal tissues (King et al., 2014). Salmon-Ehr et al (2000) showed that IL-4 may be implicated in normal wound healing (Salmon-Ehr et al., 2000). They demonstrated that topical administration of IL-4 on experimental wounds in mice significantly accelerated the rate of healing, whereas IL-4 antisense oligonucleotides significantly inhibited healing. While another study has showed that normal tissue regeneration processes after cutaneous injury were not dependent on the presence of IFN-gamma in vivo, and IL-18 serves additional roles rather than inducing IFN-gamma during the healing process (Kämpfer et al., 2000).

In order to decrease postsurgical pain, opium drugs may be used. Postsurgical pain is associated with slowed wound healing and provides additional

incentive to support aggressive pain management (McGuire et al., 2006), thus opium addiction with suppression of pain can alter the process of wound healing in surgical patients. Pain control with opioid or NSAIDs analgesics can improved wound healing (Kuang et al., 2007). Opium can decrease intra-abdominal adhesion band formation after surgery (Cassidy et al., 2010).

Almost all patients undergoing abdominal surgery develop adhesions but opium or morphine reduces the severity of postoperative adhesions (Khorram-Manesh et al., 2006). Elucidation of the opioid receptor(s) involved in this process would enable the use of selective ligands that offers a potential pharmacologic strategy in preventing adhesion formation (Khorram-Manesh et al., 2006). such studies can increase our knowledge about pathophysiology of opium addiction and benefits or side effects of opium in addicted group that underwent surgical procedure, thus in our experimental animal study, "effects of opium addiction on wound healing and post-operative adhesion" were assessed.

Materials and methods

Animals

This study was approved by the Ethics Committee at the Kerman Medical University. Experiments were conducted with male Wistar rats (200-250 g body weight; n = 20), obtained from the animal house of Kerman University of Medical Sciences. Housed conditioning was as mentioned previously (Parsania Shahrnaz et al., 2014). The rats were housed in standard plastic cages (2 rats per cage, 50 × 50 × 40 cm in size) in an environmental room at 22± 2° C and 58–65% relative humidity with a controlled 12-hour light/dark cycle. They were fed standard pellet diet and ordinary tap dextrose water (D/W5%) with or without opium supplementation.

Experimental Protocols

The animals were randomly divided into two groups: control and opium addicted groups (Opium obtained from NAJA opium agency), with 10 animals in each group. Control group had access to tap water with

D/W5% without any additive substances. In the opium group, opium dependency was induced by adding mixture of opium to D/W5% with concentration of 1-3gr/lit, (corresponding to 1-3 mg/ml). The calculated dose of opium for each rat was 35 mg/kg/day for 5 days (Asadikaram et al., 2010), followed by 2 mg/kg Naloxone (Khorram Manesh et al., 2006) injected intraperitoneally subsequent to Naloxone test. Since some of examined rats didn't show withdrawal signs, the opium dosage was increased to 80 – 100 mg/kg/day (Asadikaram et al., 2010; Condon et al., 2007) for the following days (dosage from 80-100 was increased with 10 mg/kg/day interval). Naloxane – injected rats were all kept in a transparent cage for 20 minutes and were controlled for opiate withdrawal signs. Naloxone test was repeated five days later, and it was revealed that all the rats in the opium group had withdrawal signs. Withdrawal was considered if four of the following signs was seen (Condon et al., 2007): 1-jumping (Wet dog shakes) 2- Writhing 3- Teeth chattering 4- Ptosis 5- Headshakes 6- Paw tremor. Total duration of the treatment was 33 days.

Surgical Procedure

First blood sampling from retro orbital sinus was taken with hairy tube from all rats in control group in 10th days and all rats in opium addicted group in 12th days of study after mild sedation with CO₂ (volume of blood sampling was 1cc/100gr weight in rat). Then, after induction of anesthesia via intraperitoneal Ketamine injection with dose of 100 mg/kg (Camara et al., 2008) laparotomy was done through midline incision with 3–4 cm length (from xyphoid to umbilicus) and small bowel manipulation was done. Then fascia and skin were closed in two layers separately with nylon 4-0 continuously. Three weeks after surgery, site of abdominal incision was excised elliptically and sent for wound healing grading assessment by pathologist and intra-abdominal adhesion band assessment was performed.

Wound Healing Grading

Wound healing was graded in 3 categories as inflammation, proliferation and maturation with two intermediate phases as below:

1. Inflammation markers includes: clot formation, PMN and macrophage infiltration, lack of collagen formation or new angiogenesis (Bunicard et al., 2010).
2. Proliferation markers includes: fibroblast infiltration, collagen and proteoglycans synthesis, new angiogenesis, decreased of PMN cells and granulation tissue formation (Bunicard et al., 2010).
3. Maturation markers includes: cellular and vascular depletion, scar formation (Bunicard et al., 2010).
4. In some cases intermediate phases between 1 and 2 or 2 and 3 was/were confirmed.

In both groups, site of incision excised elliptically 21 days after surgery and preserved in formalin 10% concentration, then sent for wound healing grading by pathologic examination in Dr. Dabiri lab. The pathologist was not aware what group each sample belonged to.

Post-operative intra-abdominal adhesion band assessment

In second laparotomy (21 days after first laparotomy in both groups), after excision of site, intra-abdominal adhesion in two groups was assessed. And grading was done on the basis of number, size and length of adhesion bands (Khorram Manesh et al., 2006).

Cytokine Measurement

Upon labeling, all blood samples were centrifuged in 800 g for 15 min and the plasma was taken in micro tubes and preserved in – 80 °c. Cytokines (TNF α , IFN γ and IL10) levels were subsequently measured employing/using ELISA test and their concentrations were expressed as pg/ml.

Sample size

Setting 20% as standard for mortality rate, 20 rats randomly selected for study and control groups. Three blood samples were taken from each rats.

Data presentation and statistical analysis

All computations were conducted using the SPSS

software package [version 16.0]. Chi-Square test was used for comparison between two groups. Variance analysis with repeated measure ANOVA was employed to compare serum levels of the cytokines in three stages before, immediately after, and 24h after surgery. Data are expressed as Means \pm SEM of ten animals per group. P value < 0.05 was considered statistically significant.

Results

Wound healing was assessed 21 days after surgery in both groups. Inflammatory phase was observed in about 70% of control group but proliferation phase of wound healing was seen in more than 70% of all rats in opium addicted group, without any statistical difference (table1). Post-operative intra-abdominal adhesion band was not seen in opium addicted group but in control group about 60% of all rats had at least one or two adhesion band in their intra peritoneal cavity (table 2) that was statistically significant ($p < 0.014$). In addicted group, no change was observed between measured cytokines after surgery compared with before surgery (table 3). In control group, no difference in measured serum cytokines level was found after surgery relative to before surgery ($p > 0.05$). No correlation among measured cytokines levels with wound healing and adhesion band was found.

Discussion

Our findings showed that post-operative intra-abdominal adhesion was decreased in opium addicted group, while no statistically difference was found in wound healing phase and serum levels of $TNF\alpha$, $IFN\gamma$ and IL10, in the two groups measured in all phases of examination.

As indicated by these findings; opium addiction can decrease post-operative intra-abdominal adhesions which is in agreement with other findings (Khorram Manesh et al., 2006), An imbalance between fibrin deposition and fibrinolysis during the peritoneal healing process may result in adhesion formation. Various proinflammatory mediators, and cells like phagocytes and mesothelial cells, appear to be engaged/involved in this process (Haney, 2000; Khorram Manesh et al.,

2006). Tissue injury causes the immediate onset of acute inflammation. It has long been considered that the inflammatory response is instrumental to supplying growth factors and cytokine signals that orchestrate the cell and tissue movements/mechanisms necessary for repair (Eming et al., 2007).

Our finding that either opium or morphine may prevent the formation of adhesions strongly suggests that opioid and their receptors could have an impact on adhesion formation. Although, we have not investigated the exact mechanisms through which opioids reduce the severity of adhesion formation, an overview of possible mechanisms of actions may be relevant. However, to the best of our knowledge, there are no reports on the expression/presence of opioid receptors on abdominal mesothelial cells. A possible mechanism could be the activation of axon reflexes by nociceptive stimuli to the intestinal wall, which may elicit the inflammatory cascade (Khorram-Manesh et al., 2006). Axon reflexes could be initiated due to a sensitivity to inhibition by morphine, and μ -opioid receptors have been shown to exist in peripheral endings of nociceptive nerve fibers (Khorram-Manesh et al., 2006). Increased number of mesothelial cells and circulating macrophages may lead to increased level of tissue factor, which, in turn, could lead to increased amount of thrombin and higher risk of adhesion formation. Interestingly, μ -opioid receptors have been demonstrated on (human) macrophages, and other reports have shown that morphine administration elicits macrophage apoptosis in vitro (Khorram-Manesh et al., 2006). Thus, blocking the activity of the macrophages could prevent local inflammatory processes of the peritoneum and thereby attenuate adhesion formation. After surgical trauma, macrophages are recruited to the site of damage and exhibit increased phagocytic activity. They also augment the secretion of inflammatory mediators coupled with upregulated cellular respiration, leading to an inflammatory response and also to the recruitment of new mesothelial cell (Kawanishi et al., 2013).

As another possible mechanism; an increased number of platelets may contribute to adhesion formation, and morphine, by interfering with platelet aggregation, could accelerate this process (Khorram-Manesh et al., 2006). Therefore, the net inhibitory effect of morphine

Table 1: wound healing grading between two groups 3 weeks after laparotomy.

Wound healing stage	Opium addicted group	Control group	P.V*
Inflammation	2 (20%)	7 (70%)	
Inflammation – proliferation	2 (20%)	2 (20%)	
Proliferation	5 (50%)	1 (10%)	0.057
Proliferation – maturation Maturation	1 (10%)	0 (0%)	
Add	10 (100%)	10 (100%)	

(*Chi-Square test)

Table 2: post-operative intra-abdominal adhesion band between two groups. *: P<0.05, significant differences compared to control group

Adhesion bands	Opium addicted group	Control group	P.V
.	10 (%100)	4 (40%)	
+1	·(%·)	3 (30%)	0.014*
+2	·(%·)	3 (30%)	
Add	10 (%100)	10 (%\ ··)	

(Chi-Square test)

Table 3: comparison between serum level of TNF α , IFN γ and IL10 in opium addicted group before and after surgery

cytokine	Time	Mean \pm SD	P value*
TNF α	Before surgery	207 \pm 66.6	0.106
	Immediately after surgery	161 \pm 50.7	
	24h after surgery	160 \pm 35.27	
IFN γ	Before surgery	31.7 \pm 10.14	0.164
	Immediately after surgery	29.7 \pm 5.59	
	24h after surgery	24.9 \pm 35.58	
IL10	Before surgery	6.00 \pm 1.7	0.721
	Immediately after surgery	6.70 \pm 1.54	
	24h after surgery	6.37 \pm 1.73	

*Repeated Measure ANOVA

on adhesion formation could depend on the balance between anti-, and pro-adhesion factors. Morphine could increase the expression of tissue plasminogen activator, leading to fibrinolysis and, thereby, a

reduction in adhesion formation (Khorram-Manesh et al., 2006). Ezzatabadipour et al (2011) showed that morphine reduces the number of cells in the proliferative zone and decreases the thickness of the

growth cartilage, which may alter longitudinal growth of long bones (Ezzatabadipour et al., 2011).

The immunomodulatory properties of the opioids and nociceptin are not fully understood. Regulation of immune and inflammatory responses is dependent on the functions of cytokines and many of the immunomodulatory activities of the opioids, and nociception may be due to the modulation of cytokine expression. Finley et al (2008) suggested that the regulation of cytokine, chemokine, and cytokine receptor expression is a critical component of the immunomodulatory activity of the opioids (Finley et al., 2008). Their study suggests a broad role for opioids in the modulation of the function of the immune system, and suggests possible targets for the development of new therapeutics for inflammatory and infectious diseases with a possible effect on the wound healing process. Since wound healing is a complex process which can be delayed by stress, therefore better surgical techniques and fewer surgical complications would decrease the risk of adhesion formation. Also, the effect of opium on pain control coupled with decreased catecholamine release and probably change of the level of other cytokines may be effective in post-operative adhesion formation, although more study is required for assessment of its pathophysiology and confirmation of this hypothesis.

In conclusion, although opium addiction decreased post-operative intra-abdominal adhesions but no relationship between measured cytokines (TNF α , IFN γ , IL10) and wound healing or adhesion band was found. These results may be due to effects of other unmeasured /undetected cytokines or growth factors or pain receptors on wound healing and adhesion band formation. We recommend measurement of serum level of other cytokines along with assessment of intraperitoneal and wound cytokines for the accurate diagnosis of opium addiction effects on wound healing and adhesion band formation.

Acknowledgments

The present manuscript is the product of a research project (KNRC/93-1) that was approved by the Kerman University of Medical Science. The results

described in this paper were part of MD (resident of surgery) student thesis.

Conflict of interest

The authors declare no conflict of interest. The author alone is responsible for the content and writing of the paper.

References

- Abbasizade Z, Galehdari H, Rezaei A, Seyednezhad SM, Torabi M. Evaluation of the relationship between serum levels of insulin, cortisol and growth hormones and wound healing in normal and diabetic rats. *Physiol Pharmacol* 2014; 18:92-100.
- Alizadeh AM, Sohanaki H, Khaniki M, Mohagheghi MA, Ghmami G, Mosavi M. The Effect of Teucrium Polium Honey on the Wound Healing and Tensile Strength in Rat. *Iran J Basic Med Sci* 2011; 14: 499.
- Asadikaram G, Asiabanha M, Sayadi A, Jafarzadeh A, Hassanshahi G. Impact of Opium on the serum levels of TGF- β in diabetic, addicted and addicted-diabetic rats. *Iran J Immunol* 2010; 7: 186-92.
- Bunicard FC, Anderson D, Billiv TR, Dunn DL, Hunter JG, Mathews JB, Pollack RE. In Schwartz Principles of Surgery 2010; 59: 224, The McGraw-Hill Company.
- Camara CR, Guzman FJ, Barrera EA, Cabello AJ, Garcia A, Fernandez NE, Caballero E, Ancer J. Ketamine anesthesia reduces intestinal ischemia/reperfusion injury in rats. *World J Gastroenterol* 2008; 14: 5192-96.
- Cassidy V, Uum V, Stan HM, O'Dell LE, Lutfy K, Freidman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocrin Rev* 2010; 31: 98-132.
- Condon ET, Cahill RA, O'Malley DB, Aherne NJ, Redmond HP. Evaluation of post operative peritoneal adhesion formation following perioperative Nicotine administration. *J Surg Res* 2007; 140: 135-38.
- Efron PA, Moldawer LL. Cytokines and wound healing: the role of cytokine and anticytokine therapy in the repair response. *J Burn Care Res* 2004; 25: 149-60.
- Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol* 2007; 127: 514-25.
- Ezzatabadipour M, Majidi M, Malekpour-afshar R, Eftekhavaghefi SH, Nematollahi-mahani SN. The Effects of Morphine on Tissue Structure of the Growth Plate in Male Rats. *Iran J Basic Med Sci* 2011; 14: 514.
- Finley M, Happel C, Kaminsky D, Rogers T. Opioid and nociceptin receptors regulate cytokine and cytokine receptor expression. *Cell Immunol* 2008; 252:146-54.
- Haney A. Identification of macrophages at the site of peritoneal injury: evidence supporting a direct role for

- peritoneal macrophages in healing injured peritoneum. *Fertil steril* 2000; 73: 988-95.
- Järvinen PM, Laiho M. LIM-domain proteins in transforming growth factor β -induced epithelial-to-mesenchymal transition and myofibroblast differentiation. *Cell signal* 2012; 24: 819-25.
- Kämpfer H, Paulukat J, Mühl H, Wetzler C, Pfeilschifter J, Frank S. Lack of interferon-gamma production despite the presence of interleukin-18 during cutaneous wound healing. *Mol Med* 2000; 6: 1016.
- Kawanishi K, Yamato M, Sakiyama R, Okano T and Nitta K. Peritoneal cell sheets composed of mesothelial cells and fibroblasts prevent intra-abdominal adhesion formation in a rat model. *J Tissue Eng Regen Med* 2013.
- Khaksari M, Razmi Z, Hekmat AS, Naderi V, Rostami S. The Effects of Cyclooxygenase Inhibitors on the Brain Inflammatory Response Following Traumatic Brain Injury in Rats. *Iran J Basic Med Sci* 2012; 15: 1102.
- Khorram-Manesh A, Ardakani JV, Behjati HR, Nylund G, Delbro D. The effect of opioids on the development of postoperative intra-abdominal adhesions. *Digest Dis Sci* 2006; 51: 560-565.
- Khorram Manesh A, Ardakani JV, Behjati HR, Nylund G, Delbro D. The effect of opioids on the development of post operative intra-abdominal adhesions. *Digest Dis Sci* 2006; 51: 560-65.
- King A, Balaji S, Le LD, Crombleholme TM, Keswani SG. Regenerative Wound Healing: The Role of Interleukin-10. *Adv Wound Care* 2014; 3: 315-23.
- Kuang Y, Zhu Y, Kuang Y, Sun Y, Hua C, He W. Changes of the immune cells, cytokines and growth hormone in teenager drug addicts. *Chin J Cell Mol Immunol* 2007; 23: 821.
- Labat M, Pouchelet M, Gouhier N, Boireau P, Milhaud G. Regulation by phagic T-lymphocytes of a (pluripotent?) organ stem cell present in adult human blood. A beneficial exception to self-tolerance. *Biomed Pharmacother* 2001; 55:79-90.
- Lovallo WR. The hypothalamic–pituitary–adrenocortical axis in addiction. *International J Psychophysiol* 2006; 59: 193.
- Malviya A, Negi N, Mandora M, Yadav J. Perioperative Status, Complications in Opium Addicts in Western Rajasthan. *Indian J Surg* 2011; 73: 346-51.
- Mansourian A, Askarzadeh M, Shabani M, Divsalar K. Comparison of Duration of Spinal Anesthesia with Lidocaine or Lidocaine Plus Epinephrine between Addicts and Non-addicts. *Addict Health* 2012; 4: 95-101.
- McGuire L, Heffner K, Glaser R, Needleman B, Malarkey W, Dickinson S, Lemeshow S, Cook C, Muscarella P, Melvin WS. Pain and wound healing in surgical patients. *Ann Behav Med* 2006; 31:165-72.
- Nabati S, Asadikaram G, Arababadi MK, Shahabinejad G, Rezaeian M, Mahmoodi M, Kennedy D. The plasma levels of the cytokines in opium-addicts and the effects of opium on the cytokines secretion by their lymphocytes. *Immunol lett* 2013; 152: 42-46.
- Nemati MH, Astaneh B, Safaee Ardekani G. Effects of opium addiction on bleeding after coronary artery bypass graft surgery: report from Iran. *Gen Thorac Cardiovasc Surg* 2010; 58, 456-60.
- Parsania Sh, Shabani M, Moazzami K , Razavinasab M, Larizadeh MH, Nazeri M, Asadi-Shekaari M, Moein K. Gender difference in motor impairments induced by chronic administration of Vinblastine. *Iran J Basic Med Sci In Press* 2014.
- Salmon-Ehr V, Ramont L, Godeau G, Birembaut P, Guenounou M, Bernard P, Maquart F-X. Implication of interleukin-4 in wound healing. *Lab Invest* 2000; 80:1337-1343.
- Sido B, Teklote J, Hartel M, Friess H, Buchler MW. Inflammatory response after abdominal surgery. *Best Pract Res Clin Anaesthesiol* 2004; 18, 439-54.
- Wadji MB, Rohban M, Shabani M, Bahrampour A. The effect of Opium addiction on response to major operation stress. *J Kerman Univ Med Sci* 2005; 12: 159-64.