

VITAMIN B-6 DECREASES THE INCIDENCE OF POST LAPAROTOMY INTRAPERITONEAL ADHESIONS IN WISTAR RATS

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KEYWORDS

Vitamin B6,
Intraperitoneal Adhesions,
Laparotomy

ABSTRACT:

Introduction: Despite the high prevalence and morbidity of intraperitoneal adhesions, there are no promising preventive agents to prevent adhesions. The pathogenetic basis of adhesions including inflammatory pathways, fibrinolysis, and oxidative stress provides an opportunity for the role of vitamin B6 as a preventive agent.

Objectives: This study assessed the effect of intramuscular vitamin B6 administration in the prevention of post laparotomy intraperitoneal adhesions.

Methods: This study was an experimental study with Randomized Post-test only Control Group Design, in each 10 wistar rats in the treatment and control groups. All rats were subjected to laparotomy and cecum abrasion. The treatment group received intramuscular injection of vitamin B6 10 mg/kgBB/day and the control group did not receive any injection. After 6 days, relaparotomy was performed. Determination of intraperitoneal adhesion was done based on Zuhkle Criteria. Data were collected and analysed using SPSS 26.

Results: Out of 20 samples, 13 rats had intraperitoneal adhesions, and 7 rats had no adhesions. A total of 30% of the rats in the treatment group had adhesions, much less than the control group which had 100% adhesion incidence. (HR 3.3 IK 95% (1.29-8.59), p: 0.002)

Conclusions: Vitamin B6 is a preventive agent for intraperitoneal adhesions after laparotomy. Further research through clinical trials is needed before it is applied to clinical practice.

1. Introduction

Intraperitoneal adhesions are adhesions between organs or tissues within the abdominal cavity due to abnormal fibrous tissue formed from a series of inflammatory, fibrinolysis and vascular processes (1). Approximately 95% of patients who undergo laparotomy will develop adhesions later in life. The rate of adhesion formation varies from patient to patient and depends on the type and extent of surgery performed, as well as whether or not there are postoperative complications (2).

Intraperitoneal adhesions are a major cause of morbidity resulting in multiple complications, such as bowel obstruction, chronic pain and even infertility (3). It was found that 5.7% of hospital readmissions

over a 10-year period were directly related to post laparotomy adhesions and 3.8% required operative management.

The process of intraperitoneal adhesion formation involves inflammatory mediators, fibrinolysis homeostasis and oxidative stress reactions, all of which work together to restore tissue integrity (4). Various research developments have emphasized these three mechanisms, allowing the development of other therapies that target these specific mechanisms (5).

Research using various drugs has been conducted, such as NSAIDs, anticoagulants, corticosteroids, and antihistamines, but until now there has been no effective and clinically proven way to prevent or minimize adhesions. The administration of corticosteroids, NSAIDs, heparin 3 has side effects on wound healing, fluid and electrolyte balance, and haemostatic (6).

Mechanical barriers such as lactated ringer's fluid, dextran, sodium hyaluronate, carboxymethylcellulose, and polyethylene glycol to prevent adhesion have shown promising results in experimental animals. (7). However, the use of these mechanical barriers is still controversial as peritoneal fluid is always metabolized and has been found to last only 12 hours in the peritoneum (8).

Vitamin B6 or pyridoxine, is a compound that is essential for general cellular metabolism. Vitamin B6 has been shown to modulate the effects of hypoxia and inflammation, but its local effects on the peritoneum have not been confirmed (9). Direct effects on the fibrinolysis system have not been studied. As an antioxidant, vitamin B6 provides protection against cellular damage due to oxidative stress by neutralizing ROS. Vitamin B6 content has an inhibitory effect on NO and NOS which is expected to prevent postoperative paralytic ileus. Vitamin B6 was found to suppress cytokine gene expression in macrophages by inhibiting TAK1-mediated Toll-like receptor (TLR) phosphorylation, which inhibits the subsequent NF- κ B and JNK pathways. This leads to suppression of interleukin production and increased intestinal motility which has a positive effect into the prevention of intraperitoneal adhesions (9).

The effect of vitamin B6 in preventing intraperitoneal adhesions after surgery has never been studied directly in vivo or invitro. However, the effect of vitamin B6 that can suppress all inflammatory processes that play a role in the formation of intraperitoneal adhesions through unknown pathways. In addition, this B vitamin is easily available, cheap, and can be applied to the region. Therefore, in this study, we will investigate the effect of vitamin B6 administration versus no administration on the lower incidence of intraperitoneal adhesions.

2. Objectives

This study aims to see whether intramuscular administration of vitamin B6 can prevent the incidence of intraperitoneal adhesions after laparotomy, by comparing the incidence of intraperitoneal adhesions in rats on day 6 with intramuscular administration of vitamin B6 compared to without intramuscular administration of vitamin B6 after laparotomy.

3. Methods

3.1. Research Design

This research was experimental research with *Randomized Post-test only Control Group* research design. The research was conducted at the Pharmacology Laboratory and Laboratory of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali.

3.2. Research Sample

The research samples were male Wistar rats aged 6-8 weeks with a body weight of 200-250 grams, with exclusion criteria: seriously ill or dead rats, determined by a veterinarian. The sample size was determined based on the Federer formula, obtaining a sample size of 10 rats each in the treatment group and control group.

3.3. Research Protocol

Twenty male rats (*Rattus norvegicus* strain Wistar) were prepared 1 week in advance for adaptation, maintained in wire cages measuring 20x40x40 cm. Feces were cleaned daily to prevent infection. Cages were placed in room temperature with indirect sunlight. Food was provided in the form of pelleted food every day. The health of the rats was checked daily by a veterinarian.

Rats were fed for 2 hours before surgery. General anaesthesia was injected with ketamine at a dose of 25 mg/kg intramuscularly through the thigh muscles of rats. Rats were positioned supine. Fur in the operation area was disinfected with povidone iodine 10%. The operator was the researcher himself assisted by an assistant. Laparotomy was performed along 4 cm midline. Abrasion of the cecum and parietal peritoneum directly adjacent to the cecum was performed, scraping with a gauze pad until the serous layer was injured and a 1 cm long bleeding spot was observed. The cecum was returned to its anatomical position in the abdominal cavity. The abdominal wall was closed with longitudinal sutures with 4/0 polyglycolic material, without suturing the peritoneal layer, followed by simple interrupter sutures on the skin with 4/0 silk material. The surgical wound was closed with sterile gauze and 10% povidine iodine was applied. After treatment, all experimental animals were given the same treatment in a drum in addition to the treatment under study.

Rats were randomly assigned into 2 groups; 1) Control group (K) (10 Wistar rats) Not given vitamin B-6 and 2) Treatment group (P) (10 Wistar rats) Given a daily dose of vitamin B-6 (10 mg/kg BW/day) administered intramuscularly in the thigh of Wistar rats every day for 5 days, given every 12 hours. A re-laparotomy was performed on day 6, with a U-shaped incision. Adhesion assessment based on Zuhke's intraperitoneal adhesion criteria was performed by a second surgeon, who was *blinded* as to which treatment the rats had received.

3.4. Data Analysis

The data obtained were analysed statistically by testing the comparison or difference in the proportion of adhesions in the treatment and control groups with the *Fisher Exact* test. Analysis was performed with SPSS 21.0 for Windows statistical software.

3.5. Ethical Clearance

This research protocol has obtained Ethical Clearance from the Ethics Committee of the Faculty of Medicine, Udayana University.

4. Results

This study was conducted on 10 Wistar rats in each group with and without intramuscular administration of vitamin B6. After the 6th day calculated from the day of cecal abrasion, relaparotomy was performed to macroscopically assess the incidence of peritoneal adhesions (based on Zuhkle's classification), All rats were alive and no samples were *dropped out*.

4.1. Incidence and Degree of Intraperitoneal Adhesions Based on Zuhkle Classification in Treatment and Control Groups

Of the 20 rats, 13 rats had intraperitoneal adhesions. 10 of them were control group rats, and 3 from the treatment group. Of the rats that experienced adhesions, the majority of adhesions were moderate (grade 3) as shown in Table 1.

Table 1. Distribution of incidence and degree of intraperitoneal adhesions based on Zuhkle's classification in treatment and control groups

Degree of Adhesion	Treatment Group	Control Group	Total
No adhesion (Grade 0)	7 (70%)	0 (0%)	7 (35%)
Adhesion			
-Thin (Grade 1)	1 (10%)	2 (20%)	3 (15%)
-Light (Grade 2)	0 (0%)	1 (10%)	1 (5%)
-Moderate (Grade 3)	2 (20%)	5 (50%)	7 (35%)
-Heavy (Grade 4)	0 (0%)	2 (20%)	2 (10%)

4.2. Difference in Intraperitoneal Adhesion Incidence of Treatment Group and Control

To determine differences in the incidence of intraperitoneal adhesions in the treatment and control groups in Wistar rats, a statistical test of categorical data was performed. Because the data in this study had a freedom value of less than 5, the statistical test used was the Fischer Exact Test. The statistical test results showed that there was a significant difference in the incidence of intraperitoneal adhesions between the treatment and control groups ($p=0.002$). Intramuscular administration of vitamin B6 reduced the risk of adhesion 3.3 times compared to no administration of vitamin B6 (Table 2).

Differences in the incidence of intraperitoneal adhesions in the control and treatment groups of Wistar rats.

Group	Adhesion		HR (95%CI)	P
	Yes	No		
-	3(30%)	7(70%)	3.3	0.002
Application	10(100%)	0(0%)	(1,29-	
-Control	13 (65%)	7(35%)	8.59)	
-Total				

5. Discussion

The peritoneum is a thin mucosa that lines the abdominal cavity and covers the intra-abdominal organs. The peritoneum consists of a single layer of mesothelial cells and a loose stroma of connective tissue and fibroblasts (10). Triggers for adhesion formation include inflammation, endometriosis, chemical peritonitis, radiotherapy, foreign body reaction, and ambulatory peritoneal dialysis, but most adhesions are induced by surgery (11). Peritoneal adhesions are reported to be the cause of 65-75% of obstructed ileus cases, and half of these are acute. It is estimated that peritoneal adhesions develop after 93-100% of upper abdominal laparotomies and 67-93% of lower abdominal laparotomies (12). In a meta-analysis, it was reported that the mean rate of intraperitoneal adhesions after laparotomy was 54% (95%CI 40-68%), more specifically 66% (95%CI 38-94% after gastrointestinal surgery, 51% (95%CI 40-63%) after obstetric and gynaecologic surgery and 22% (95%CI 7-38%) after urologic surgery (13). The interval between laparotomy and the first episode of obstructive ileus varies from eight days to 60 years with a mean interval of 3.7-8.9 years (12).

In this study, the overall incidence of intraperitoneal adhesions was 13 out of 20 rats subjected to cecal abrasion (65%). In the group that was not given vitamin B6 supplementation, all of them had intraperitoneal adhesions. In the group that received vitamin B6 supplementation, only 30% of Wistar rats experienced intraperitoneal adhesions. However, there is a need to better understand the pathophysiology of intraperitoneal adhesion formation that will underlie the clinical bridge for the application of safe and effective therapeutic options in the prevention of intraperitoneal adhesions.

The results of this study provide quantitative evidence of the role of vitamin B6 supplementation in preventing intraperitoneal adhesions. In this study, it was shown that the incidence of intraperitoneal adhesions in the treatment group was significantly lower than the control group ($p=0.002$), where no non-adhesion events were found in wistar rats that did not receive vitamin B6 supplementation. In risk analysis, intramuscular vitamin B6 administration was found to reduce the risk of adhesion 3.33 times compared to no vitamin B6 administration.

Vitamin B6 theoretically has interesting biological properties and actions for the prevention of peritoneal adhesions. In vitro studies show that this vitamin has antioxidant, anti-inflammatory, anticoagulant, and ant fibroblastic effects, and decreases collagen production. However, it is not well studied.

Tissue hypoxia can contribute to increased oxidative stress with increased generation of nitrogen and oxygen free radicals, leading to DNA damage and increased production of oxidized proteins (14). During the first 5 minutes after hypoxia, free radicals are produced significantly through increased

formation of reactive oxygen species (15). ROS can induce the activation of transcription factors that can activate the activity of MMPs, which induces adhesion (16). This study has shown that vitamin B6 reduces oxidative stress through the NO pathway, which bridges a potential effect for adhesion prevention.

The severity of inflammation is associated with increased adhesion formation. Although genes involved in ECM deposition are decreased within 24 hours after injury, including TGF, fibronectin, and collagen, the increased production of inflammatory mediators at an early stage plays an important role in regulating ECM formation during PA (17). Both TNF- α and IL-6 play a role in regulating the formation of the coagulation cascade and fibrin formation, so a decrease in both would be an important factor in the prevention of adhesion formation (18). This study has shown that vitamin B6 reduces inflammation through the IL-6 pathway, which bridges the potential effect for adhesion prevention.

Extensive tissue injury, hypoxia and inadequate fibrinolytic activity of the peritoneum contribute to an imbalance between procoagulation and fibrinolytic reactions, inducing fibrin clot formation. Under normal conditions, fibrin bands can be degraded into smaller molecules by fibrinolysis regulated by the enzyme plasmin. Plasmin is derived from urokinase-like plasminogen activator and tissue-type plasminogen activator (tPA). While to maintain balance, tPA can be regulated by plasminogen activator inhibitor-1 (PAI-1) (19). Severe peritoneal trauma in laparotomy causes an imbalance between tPA and PAI-1 with the result of increased fibrin exudate and fibrin mass, leading to intraperitoneal adhesions (18) This study has shown that vitamin B6 reduces fibrinolysis through the PAI-1 pathway, which bridges the potential effect for adhesion prevention.

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