

The Effect of an Intraperitoneal Injection of Melatonin on Serum Amylase Levels in Acute Pancreatitis

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ABSTRACT

Context Several experimental studies have been carried out to explain the physiopathological mechanisms and to introduce endocrinological, enzymatic, biochemical and histopathological changes in organisms during acute pancreatitis. **Objective** To evaluate the effect of an intraperitoneal injection of melatonin on serum amylase levels. **Design** Experimental acute pancreatitis was experimentally caused through pancreatic duct ligation in 20 Wistar Albino rats. The rats were then divided into two groups: control and melatonin groups. **Intervention** The serum amylase level was measured on the 7th day after acute pancreatitis had developed. In the melatonin group, an intraperitoneal injection of melatonin (20 mg/kg/day) was performed starting from the 2nd day after pancreatic duct ligation. **Main outcome measure** The levels of serum amylase were measured with an auto analyzer. **Results** It was found that the mean (\pm SD) level of serum amylase in the control group was 947 \pm 182 IU/mL while it was 358 \pm 177 IU/mL in the experimental group ($P < 0.001$). **Conclusions** The 20 mg/kg/day intraperitoneal injection of melatonin which was carried out for one week attenuated the serum amylase levels to a statistically significant degree. The researchers believe that intraperitoneal injections of melatonin decrease the severity of acute pancreatitis.

INTRODUCTION

Acute pancreatitis is a disease which develops with perivascular infiltration and inflammation characterized by lipid necrosis, polymorphonuclear leukocyte infiltration, hemorrhage, acinar cell necrosis and tissue edema in the pancreas.

Acute pancreatitis is still a serious disease as its diagnosis and treatment is difficult [1, 2]. It is usually accompanied by mild and restricted clinical conditions; however, necrotizing acute pancreatitis may sometimes result in a serious outcome. Despite current intensive care techniques, the development of nutritional support applications, fluid-electrolyte replacement and mechanic ventilation facilities, the mortality rate may reach 10-20% [1].

Studies published in the literature concerning acute pancreatitis demonstrate that, in the first 24 hours, many morphological changes, such as lipid necrosis in pancreatic tissue, hemorrhage and inflammation

occurred in test subjects with occluded pancreatic ducts [3]. In the earlier and later stages of acute pancreatitis, proteolytic enzymes become active after the extravasation of pancreatic secretions into the interstitial space. Inflammation becomes more severe because of edema in the tissue, disruption of the microcirculation and cell ischemia; thus, toxic mediators start to accumulate in the tissue [4].

Two enzymes (amylase and lipase) are released from the acinar cells during acute pancreatitis and their concentration in the serum is used to confirm diagnosis. Serum amylase concentrations exceeding three times the normal upper limit support the diagnosis of acute pancreatitis. The specificity of the diagnostic value an increased amylase level is approximately 95%. It is therefore widely used in the diagnosis of acute pancreatitis and the assessment of its severity as it is considered an important indicator of the disease [4, 5, 6].

Melatonin is a hormone which affects the general immune system and cellular immunity both directly and indirectly. Melatonin, which is synthesized within pinealocytes in the pineal gland and secreted rhythmically in accordance with daylight, can overcome all the morphophysiological barriers in the human body and can reach the intercellular area in all organs and cell nuclei easily due to its lipophilic and hydrophilic features [7].

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Melatonin is secreted by the intestines as well as by the pineal gland; however, 90% of the melatonin in the body is secreted by the pineal gland [7, 8]. The number of studies carried out regarding the effect of melatonin on amylase values in test subjects who have experimental acute pancreatitis and a high level of amylase is quite limited. The aim of this study was to address the effects of exogenous melatonin on the amylase level in acute pancreatitis. For this purpose, over the period of one week, melatonin (20 mg/kg/day) was injected intraperitoneally into rats with experimentally acute pancreatitis which had been developed through ligation of the pancreatic duct; the differences and changes which occurred in the serum amylase levels were examined in detail and the results were compared statistically with those of the control group.

MATERIALS AND METHODS

In this experimental study, 20 adult Winstar Albino male rats (average weight 200-250 g) were used. The test subjects were divided into two groups of 10 rats each. In the 10 control rats, only experimental acute pancreatitis was caused and they received no other treatment. In the 10 rats of the experimental group, in addition to having acute pancreatitis caused experimentally, intraperitoneal melatonin (20 mg/kg/day) was injected every day for one week. At the end of one week, blood samples were taken from the test subjects and the test subjects were then sacrificed.

The surgical and medical procedures performed on the experimental animals were carried out under general anesthesia and standard sterile conditions. General anesthesia was administered with 20 mg/kg Ketamin HCl plus 5 mg/kg Xylazine HCl (i.m.) and prophylactic antibiotics were used in all animals.

Acute pancreatitis was provoked through surgical ligation of the common pancreatic duct by a 2 cm midline laparotomy. The pancreas was explored and the procedure finalized with duct ligation using 5/0 polypropylene suture.

In the test subjects, 24 hours after the acute pancreatitis was developed, melatonin (Sigma, St. Louis, MO, U.S.A.) 20 mg/kg/day was injected intraperitoneally for one week everyday between 06:00 and 08:00 pm. During the study, one test animal in each group died due to technical complications and problems related to the dose of anesthesia; thus, the study was carried out with 9 test subjects in each group. At the end of the 7th day, 2 mL blood samples were taken from the subjects by means of intracardiac puncture, and were stored in plain biochemistry tubes without anticoagulants (Becton Dickinson, Franklin Lakes, NJ, U.S.A.).

Twenty minutes after withdrawal, the blood samples were centrifuged at 3,500 g for 15 minutes and the serum collected through centrifuging was diluted with isotonic NaCl and the amylase levels were measured with an auto analyzer. Abbott Architect C8000(Chicago, IL, U.S.A.) was used as an amylase kit and the values were recorded in IU/mL.

ETHICS

This experimental animal study was conducted in accordance with the principles and procedures outlined in the "NIH Guide for the care and use of animals" and carried out with ethical approval (Decision number; 2008/116) obtained from the Animal Care Ethics Committee of Abant Izzet Baysal University Medical School.

During the study, all the experimental animals were kept at normal room temperature (22°C) in a 12-h light and 12-h dark cycle and were fed with standard pellet rat food (210 kcal/100 g/day) and tap water.

STATISTICS

Statistical analysis was carried out using the SPSS-11 for Windows program (SPSS Inc., Chicago, IL, U.S.A.). Amylase levels, shown as mean \pm standard deviation (SD), were analyzed by means of the Student's t-test. A two-tailed P value of less than 0.001 was considered as statistically significant.

RESULTS

Before starting the experimental studies, the pancreatic tissue of all the test subjects in both groups was determined to be healthy and the structure of the ducts was normal after laparotomy. Normal samples of pancreatic tissue were taken from a few of the test

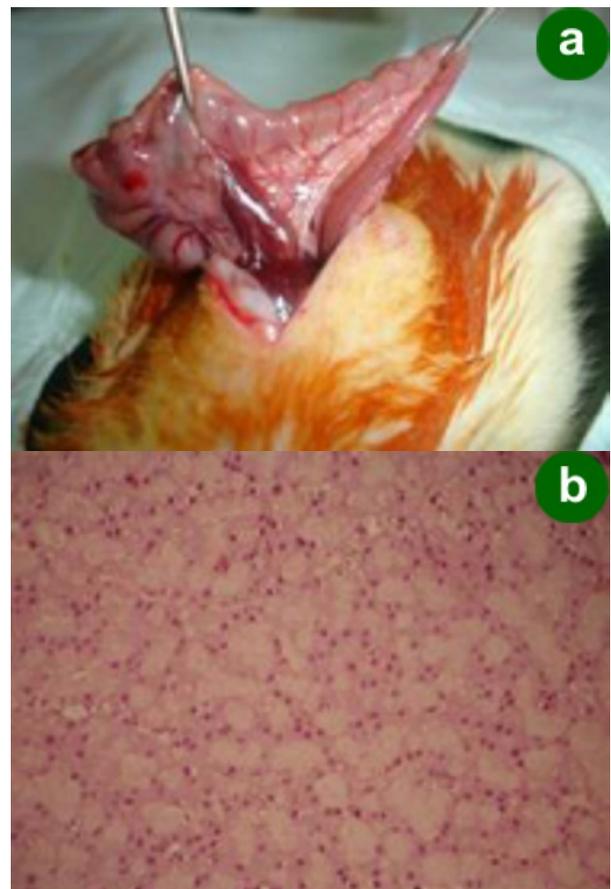


Figure 1. a. The macroscopic appearance of a normal pancreas and duct structure in rats. **b.** Normal pancreas tissue in rats at light microscopy (H&E x40).

Table 1. The serum amylase levels (IU/mL) measured in the subjects.

Subjects	Control group	Melatonin group
#1	1,262	405
#2	1,056	181
#3	1,186	393
#4	717	271
#5	846	468
#6	851	116
#7	844	475
#8	826	684
#9	931	225
Mean± SD	947±182	358±177
	t=6.95; P<0.001	

subjects and examined for comparison and histopathological assessment; however, these test subjects were not used in the experimental study (Figure 1).

When re-laparotomy was performed at the end of the experimental study, findings of dilatation in the pancreatic duct as well as edema and inflammation in the pancreas were observed macroscopically (Figure 2a); after taking a blood sample by intracardiac puncture to measure the serum amylase level, the samples of pancreatic tissue were taken for histopathological tests. In the microscopic investigation of the pancreas tissue, polymorphonuclear cell infiltration, edema, inflammation and ductal dilatation were observed (Figure 2b).

The serum amylase levels at the end of the 7th day of the experimental study were found to have a minimum of 717 IU/mL and a maximum of 1,262 IU/mL in the rats of the control group (Table 1). The average serum amylase was 947±182 IU/mL. On the other hand, it was found that, in the melatonin group, the amylase levels ranged between 116 and 684 IU/mL, and the average value was determined to be 358±177 IU/mL. The difference between the amylase levels of the control and melatonin groups was statistically significant (t=6.95; P<0.001).

DISCUSSION

Figure 1 shows the consistency of the macroscopic and microscopic appearance before acute pancreatitis with the normal pancreatic tissue. The observation of duct dilatation along with edema in the pancreas macroscopically after experimental acute pancreatitis was developed is given in Figure 2. The microscopic determination of the histopathological findings of acute pancreatitis proves that acute pancreatitis was successfully developed after the ligation of the pancreatic duct.

In the subjects of the control group, the fact that the serum amylase level at the end of the 7th day varied between 717 and 1,262 IU/mL (mean±SD: 947±182 IU/mL) was considered to be a biochemical indicator showing that experimental acute pancreatitis had been developed successfully. The increase in amylase level (358±177 IU/mL) in the group which was given exogenous melatonin was less than half of the levels in the control group. According to this finding, it can be concluded that using exogenous melatonin prevents

amylase values from increasing more than necessary or decreases a serum amylase level which was already too high.

Melatonin is a hormone which can be found in body fluids such as cerebrospinal fluid, saliva and bile; it is not stored and is rapidly released into the general circulation immediately after being synthesized by the pineal gland. The concentration of melatonin in these body fluids is almost 2-3 times higher than its concentration in serum [9]. The night-time secretion of melatonin takes place in the pineal gland while it is secreted by gastrointestinal system mucosa during the day, especially after the intake of food.

Melatonin does not undergo auto-oxidation, redox cycling or reactions producing hydroxyl radicals. Therefore, in contrast to other antioxidants, it does not have a toxic effect even if it is used in very high doses (300 mg/day) and for a long time [10].

Melatonin, which can overcome all body barriers and the placenta, can easily be effective for all intracellular components. Thus, melatonin can protect the cell wall, organelles and cell nucleus from damage by free radicals [8, 11]. Deactivation of melatonin occurs mainly in the liver by microsomal enzymes which metabolize melatonin into 6-hydroxymelatonin.

It has previously been shown that normal serum amylase levels in healthy rats on a standard diet are

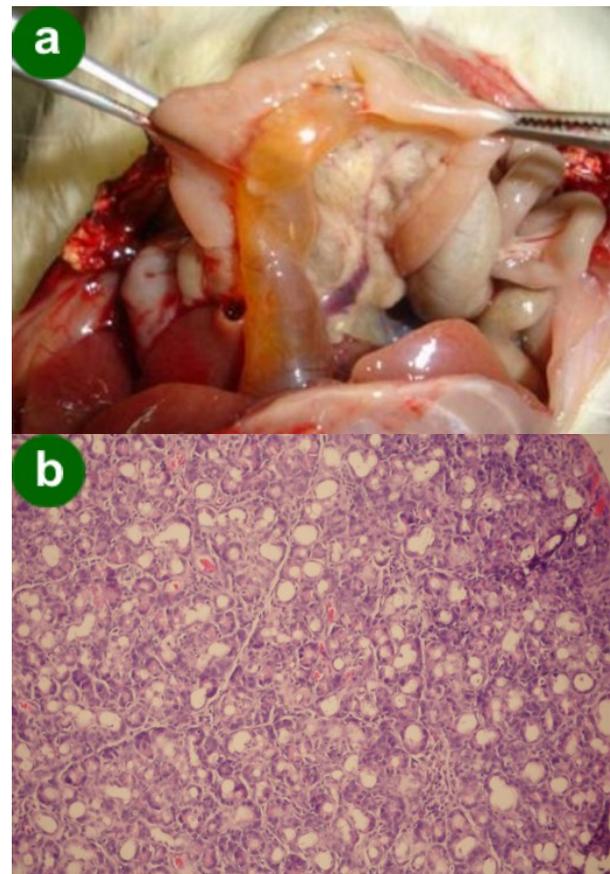


Figure 2. a. The macroscopic appearance of acute pancreatitis caused by duct ligation. There is very clear extreme dilatation in the choledochus and edema in the pancreatic tissue. b. Histopathological findings of acute pancreatitis in test subjects (H&E x40).

approximately 250 IU/mL, and 65 IU/mL after 12 h fasting [12]. Pancreatic enzyme activity in serum is changed with feeding and pancreatic functioning. The question in the current study was to evaluate whether or not the melatonin had any effect on serum amylase levels. The results of published studies carried out to investigate the effect of melatonin on amylase level differ from each other. Melatonin has been shown to decrease the level of amylase in some studies whereas it has increased it in others [2, 13, 14, 15, 16]. In the rats with a pancreaticobiliary fistula, intraperitoneal administration of melatonin significantly stimulated serum amylase concentrations. Despite the *in vivo* observations demonstrating that melatonin is a potent pancreatic secretagogue, none of above substances was able to directly affect amylase release from isolated pancreatic acini [14, 16]. A large number of experimental studies have been carried out to demonstrate the protective effect of different doses of melatonin on tissue damage caused by ischemia reperfusion injuries in the liver, kidney and intestines, in addition to burns, compressed injuries and certain medications [17, 18, 19].

Giving exogenous melatonin to healthy organisms resulted in a considerable increase in amylase activity [2, 13, 15]. It has been demonstrated that melatonin decreases lipid peroxydation and tissue edema in experimental acute pancreatitis and thus causes the amylase activity to decrease [13, 14]. As is seen, the results regarding the effects of melatonin on amylase levels vary to a great extent. In the current study, it was found that melatonin decreased the elevated level of amylase in rats with pancreatitis. In considering the results of experimental studies carried out previously and the data obtained in the current study, it can be concluded that injections of exogenous melatonin increase amylase levels in healthy organisms while they help to reduce elevated amylase levels due to acute pancreatitis.

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Conflict of interest The authors have no potential conflicts of interest

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