The Effects of Long Term Ketamine Use on Pancreatic Islet Cells

Uzun Süreli Ketamin Kullanımının Pankreas Adacık Hücreleri Üzerine Etkileri

Habib BOSTAN,[a]
Yakup TOMAK,[b]
Deniz ÜNAL,[c]
Levent TÜMKAYA,[d]
Durdu ALTUNER,[e]
Adnan YILMAZ,[f]
Orhan BAŞ,[g]
Yıldıray KALKAN,[d]
Adem KARA,[c]
Başar ERDİVANLI,[h]
Jale SELLİ,[c]
Alparslan TURAN[i]

[a]The Ministry of Justice, Council of Forensic Medicine, İstanbul
[b]Department of Anesthesiology and Reanimation, Sakarya University Faculty of Medicine, Sakarya
[c]Department of Histology, Atatürk University Faculty of Medicine, Erzurum
Departments of
[d]Histology,
[e]Pharmacology,
[f]Biochemistry,
[h]Anesthesiology and Reanimation, Recep Tayyip Erdoğan University Faculty of Medicine, Rize
[g]Department of Anatomy, Ordu University Faculty of Medicine, Ordu
[i]Department of Outcomes Research, Cleveland Clinic, Cleveland/USA

Geliş Tarihi/*Received:* 01.11.2012
Kabul Tarihi/*Accepted:* 16.05.2013

Yazışma Adresi/*Correspondence:*
Habib BOSTAN
The Ministry of Justice, Council of Forensic Medicine, İstanbul, TÜRKİYE/TURKEY
habibbostan@yahoo.com

doi: 10.5336/medsci.2012-32420

ABSTRACT Objective: Ketamine is a frequently used anesthetic with a prominent effect on blood glucose levels. Therefore, ketamine may alter the mode of action of pancreatic islet cells, and the prolonged use of this agent may cause functional or morphological alterations of the pancreatic tissue. This study compared prolonged and different doses of intraperitoneal ketamine administration on pancreatic islet cell morphology and secretory function in rats. **Material and Methods:** A total of 30 rats in five groups were used. Rats in groups K40, K60, K80 and K100 received 40, 60, 80, and 100 mg/kg ketamine hydrochloride intraperitoneally (i.p.), respectively. Control group received 0.9% NaCl i.p.. Injections were repeated twice daily for 2 weeks. After 2 weeks, the animals were sacrificed under anesthesia, pancreas tissues were removed and examined using immunohistological staining, light microscopy, and electron microscopy. **Results:** Prolonged use of ketamine caused histological alterations as observed by electron microscopy in all groups and by light microscopy in all groups except K40. Immunopositivities for insulin and glucagon was statistically significantly higher in the control group compared to study groups, but did not differ between study groups. **Conclusions:** We demonstrated that the long-term ketamine use had pathological effects on the pancreas histology. Therefore, it should be kept in mind that ketamine causes pancreas damage and should be used cautiously. Further investigations are needed to find out whether these effects are permanent or not.

Key Words: Pancreas; microscopy, electron; ketamine; immunohistochemistry

ÖZET Amaç: Ketamin sık kullanılan bir anestezik olup, kan glikoz düzeyi üzerinde belirgin etkisi vardır. Uzun süreli ketamin kullanımında pankreas adacık hücre morfolojisi ve salgı fonksiyonu değiştirebilir. Bu çalışmada, ratlarda farklı dozlarda ve uzun süreli ketaminin intraperitoneal (i.p.) kullanımının pankreas adacık hücre morfolojisi ve salgı fonksiyonu üzerine etkisi karşılaştırıldı. **Gereç ve Yöntemler:** Beş grupta 30 adet rat kullanıldı. Gruplar K40, K60, K80 ve K100 olarak düzenlendi ve ratlara sırasıyla 40, 60, 80 ve 100 mg/kg ketamin hidroklorür i.p. olarak verildi. Kontrol grubuna ise %0,9 NaCl i.p. uygulandı. Enjeksiyonlar 2 hafta süreyle ve günde iki kez tekrarlandı. Ratlar iki hafta sonra anestezi altında sakrifiye edilerek, pankreas dokuları çıkartıldı. Elde edilen dokular, ışık mikroskobu, elektron mikroskobu ve immünohistolojik boyama yöntemleri kullanılarak incelendi. **Bulgular:** Tüm uygulama gruplarında elektron mikroskobu ile histolojik değişiklikler tespit edildi. Aynı zamanda K40 hariç tüm gruplarda yapılan ışık mikroskobik incelemelerde histolojik değişiklikler gözlendi. Çalışma grupları ile kontrol gruplarının insülin ve glukagon immünpozitiflikleri karşılaştırıldığında, çalışma gruplarında istatistiksel olarak anlamlı derecede yüksek bulundu. Ancak çalışma grupları arasında fark yoktu. **Sonuç:** Bu çalışmada uzun süreli ketamin kullanımının pankreas dokusunda patolojik etkilere neden olabileceğini gösterdik. Uzun süreli ketamin kullanılan hastalar pankreas yetmezliği belirtileri açısından da takip edilmelidir. Ketaminin pankreas üzerine olan bu etkilerinin kalıcı mı yoksa geçici mi olduğunu söyleyebilmek için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Pankreas; mikroskopi, elektron; ketamin; immünohistokimya

Turkiye Klinikleri J Med Sci 2013;33(4):1088-96

Ketamine is a dissociative anesthetic with a significant analgesic effect. Unique properties like minimal depression of respiration and stimulation of cardiac functions causes ketamine to be the preferred anesthetic in certain cases. These include surgical interventions where anesthesia personnel and monitoring equipments are limited, component of multimodal analgesia and during dressing changes in burn patients.[1-3] If prolonged action is required, multiple doses or ketamine infusion are used. Infusion is often used in chronic pain management usually in the treatment of Complex Regional Pain Syndrome (CRPS)[4-6] and for the treatment of withdrawal from alcohol addiction.[7,8] In addition, multiple injections of ketamine is also very common in recreational use.[9,10] Ketamine and xylazine have significant impact on blood glucose levels.[11,12] Effect on blood glucose regulation is complex, demonstrating both hyperglycemic and hypoglycemic properties. Decrease of insulin secretion and glycogenolytic effects of ketamine are through its effects on circulating catecholamines and the adrenergic innervations of the pancreas and liver.[13] Consequently, long-term ketamine use has been associated with hepatotoxicity and structural changes in CRPS patients.[14] Furthermore, ketamine is shown to reduce pancreatic blood flow in animal studies.[15]

There are, thus, compelling reasons to believe that overstimulation of pancreas by long-term ketamine use or by reducing pancreatic blood flow might cause changes in the pancreas tissue. However, there have been no reports in the literature concerning the histopathological changes in pancreas following prolonged ketamine use.

Our primary goal was to evaluate the effect of prolonged and varied doses of i.p. ketamine administration on pancreatic islet cell morphology and secretory function in rats. Pancreatic tissues were examined using multiple techniques including immunohistological staining, light microscopy, electron microscopy (EM) and immunohistological staining.

MATERIAL AND METHODS

The protocol of this study was reviewed and approved by the Local Ethics Committee for Animal Experiments, School of Medicine, Rize University, Turkey (date: 31.05.2010, meeting number: 17).

ANIMALS

Thirty Wistar albino male rats, 210 - 270 days old, weighing between 250-300 g, born and raised at the Veterinary Control and Research Institute, Erzurum, Turkey were used in the study. All animals were fed with standard 7-8 mm pelleted rat diet (Erzurum, Turkey) and water ad libitum. Room temperature and humidity were preserved at 20±3 ^{0}C and 55-60% respectively, with 15 air changes per hour. Fluorescent lighting was provided automatically by a 12-h light (07:00 to 19:00) and a 12-h dark period. Experiments for animal studies were performed according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

ANESTHETIC METHOD

A total of 30 rats were divided into 5 groups (n=6). Rats in groups K40, K60, K80 and K100 received 40, 60, 80, and 100 mg/kg ketamine hydrochloride (Parke Davis & Co, UK) i.p., respectively. The control group C received 0.9% NaCl i.p. The injections were repeated twice daily, at 08:30 and 16:00, for 2 weeks. At the end of two weeks, the animals were sacrificed under ketamine anesthesia and pancreas tissues were removed and processed for histopathological examination.

The immunoreactivity of the tissues was graded by four histologists and one anatomist who were blinded to the treatments using a three point scoring system graded as weak (+), moderate (++) and intense (+++).[16]

PROCESSING THE TISSUES FOR ELECTRON MICROSCOPY

Pancreas tissue specimens were intracardially perfused by 0.9% saline NaCl (30 ml) solution followed by a mixture of 2% paraformaldehyde + 2% glutaraldehyde (150 ml) in 0.1 M phosphate buffer at a pH of 7.4 and at room temperature for 2 hours,

and subsequently at 4^0C overnight. The following day, the specimens were fixed in 1-2% osmium tetroxide for 1 h. After tissue dehydration through a graded series of ethyl alcohols, the sections were blocked and embedded in Epoxy Resin KIT (AGAR 100®; Agar Scientific, U.K).[8] The semi-thin sections of the specimens were cut at a thickness of 1 μm for toluidine blue staining. Ultra thin sections with a gold impediment color were obtained using an ultramicrotome (LXB 2188 Ultramicrotome, NOVA, Bromma Sweden) and monitored using a JEM-100S electron microscope (Jeol, JEM-100SX Electron Microscope, Tokyo, Japan) operated at an accelerating voltage of 80 kV. Afterwards, electron micrographs of the specimen were taken at a magnification of 3000X and 6000X for examination. Electron micrographs were taken by using an electron microscope (EM 9 Carl Zeiss, Germany) in Erzurum,Turkey.

LIGHT MICROSCOPIC HISTOPATHOLOGICAL EXAMINATIONS

Pancreas tissues excised for light microscopic examination were fixed in 10% neutral buffered formalin fixative solution for 24 h. After dehydration, in a series of ethanol and xylene solutions, tissues were embedded in liquid paraffin, sectioned between 4-5 μm using a rotary microtome and stained with hematoxylin-eosin.[17] Light microscopic examinations were used to confirm definition of pancreatic cells.

LIGHT MICROSCOPIC IMMMUNOHISTOCHEMISTRY

The sections, 4-5 μm thick, were de-paraffinized in xylene for 20 minutes. The slides were air-dried, and progressively dehydrated in graded ethanol solutions (70-99%). The sections were dipped in 3% H_2O_2 in distilled water for 10 minutes at room temperature to block endogenous peroxidase activity. Phosphate buffered saline (PBS) (pH=7.4) was used for all subsequent washes. The slides were heated in a microwave oven (400 watts) for 20-30 minutes in citrate buffer (pH=6.0). After nonspecific binding was blocked with blocking reagent for 5 minutes, primary antibodies, anti-insulin (Flex, Polyclonal Guinea Pig Anti-insulin, IR002, Dako, USA) and anti-glucagon (Polyclonal Rabbit Anti-Human Glucagon, A0565, Dako, USA) were applied at ratios of 1:100 for insulin and 1:75-1:100 dilutions for glucagon at room temperature for 60-75 minutes. Subsequent steps were performed in accordance with the universal kit that was used with the rabbit/mouse streptavidin biotin technique (DBS). Visualization of the bound primary antibodies was performed with diaminobenzidine solution (Sigma Chemical Co., St. Louis, MO, USA) as a chromogen and counterstained with Mayer's hematoxylin. PBS alone was used as negative control. Slides that were shown previously to be strongly positive for insulin and glucagon were used for each antibody as positive controls.

STATISTICAL ANALYSIS

The statistical analysis was performed using SPSS (IBM SPSS Statistics 18.0, IBM Corporation, Somers, NY, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests showed that none of the data showed a normal distribution. Therefore, all groups were compared using Kruskal-Wallis test. Groups were separately compared using Mann-Whitney-U test (post hoc analysis using Bonferroni's correction). The p value is obtained by dividing 0.05 by ten of the comparison number. A p-value less than 0.005 was considered significant (≤0.005).

RESULTS

ELECTRON MICROSCOPIC HISTOPATHOLOGICAL EXAMINATIONS

Electron microscopic section of tissues was examined by four histologists and one anatomist. Electron microscopic examination of the control group revealed normal exocrine pancreatic acinar cells with regular nuclear membrane contour, healthy endoplasmic reticulum, and healthy mitochondria. In addition, the acinar cells, some of which had excreted their contents, the capillary lumen in the connective tissue, and the nuclei of endothelial cells were normal (Figure 1A, Figure 2A).

Examination of *group K40* revealed that exocrine pancreatic acinar cells contained two irregularly shaped nuclei (Figure 1C, arrow), and numerous cytoplasmic zymogene granules. Some of these granules and mitochondria were damaged. Some of the condensing vacuoles (arrows) were as-

sociated with the Golgi complex (Figure 1B, C). Cells in some ducts were dilated and intracellular edema was evident.

Examination of tissues from *group K60* revealed that the nuclei of some **centroacinar cells** had irregular nuclear membranes. In addition, condensing vacuoles (open white arrows) and damaged nuclear membranes (arrow) were observed (Figure 1D). Some zymogene granules were damaged. Irregular capillary lumen and peri-nuclear edema was seen in the nuclei of the endothelial cells (Figure 2B).

Examination of *group K80* sections revealed a narrowed capillary lumen (Figure 2C), irregular nuclei and cytoplasm, some discharge of the contents of zymogen granules, damaged mitochondrial membranes (arrow), irregular nuclear membranes (white arrow), and a dilated endoplasmic reticulum (Figure 1E).

Examination of *group K100* revealed an irregular capillary lumen (Figure 2D), vacuolization of the endothelial cell cytoplasm, irregular nuclear membranes (arrow), mitochondrial membrane damages (white open arrow), and dissociated cellular junction in the cytoplasm of the pancreatic exocrine acinar cell (white arrow) (Figure 1F).

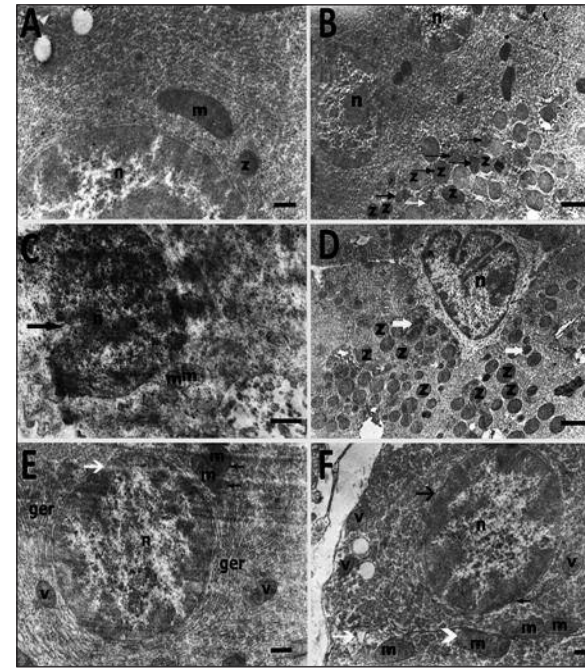

FIGURE 1: Pancreatic slices of different groups; **A:** Exocrine pancreatic acinar cell section of the control group, bar 400 nm, **B:** Exocrine pancreatic acinar cell section of Group I, bar 160 nm, **C:** Section of Group I, bar 400 nm **D:** Group II section, bar 1500 nm, **E:** Centroacinar cell section of Group III, bar 600 nm, **F:** Exocrine pancreatic acinar cell section of Group IV, bar 600 nm. m: Mitochondria; z: Zymogen granule; ger: Granule endoplasmic reticulum; n: Nucleus. (Uranyl acetate and lead citrate staining.)

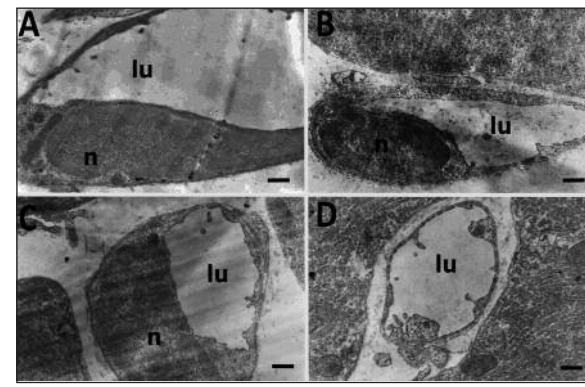

FIGURE 2: Pancreatic capillary of different groups; **A:** Capillary section of control group, bar 750 nm, **B:** Group II capillary section, bar 600 nm, **C:** Group III capillary section, bar 600 nm **D:** Group IV section, bar 600 nm. lu: Capillary Lumen; n: Capillary nucleus. (Uranyl acetate and lead citrate staining).

RESULTS OF LIGHT MICROSCOPIC IMMMUNOHISTOCHEMISTRY AND HISTOPATHOLOGICAL EXAMINATIONS

Histopathologically, *group K40* was similar to the control group for the distribution and quantity of the zymogen. The acinar cells that were binucleated, showed either central or random distribution. Some capillaries were slightly dilated due to edema (Figure 3). In semi-thin sections that were stained with toluidine blue, the vascular endothelial cells were slightly swollen (Figure 4). The islets were evenly positive for insulin and glucagon by immmunohistochemistry (graded as +) (Table 1, (Figures 5 and 6).

Group K60 sections showed slight cellular degeneration. Capillary dilation was more pronounced than the control and first group. There was also swelling and dilation of the endothelial cells. In semi-thin sections, the distribution of the zymogen granules was irregular. There was a slight degeneration and vacuolation of the acinar cells. Vacuolation was also evident in the Langerhan's cells (Figure 4). The islets were evenly positive (++) for insulin and glucagon by immmunohistochemistry (Table 1), (Figures 5 and 6). The distribution of in-

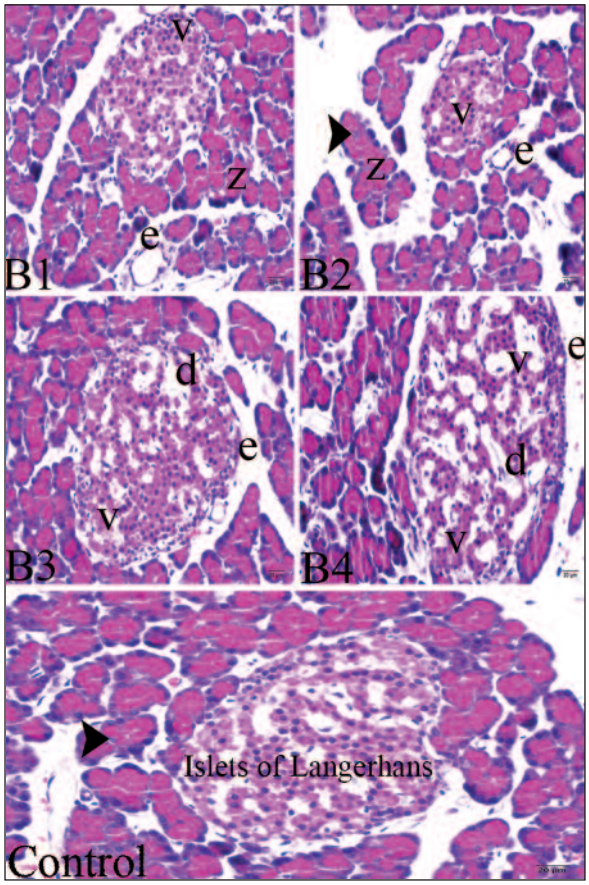

FIGURE 3: B1, B2, B3, B4 and Control: Micrographs of sections stained with HE in pancreas. Zymogene granules (Z), acinus, acinar cell (a), centroacinar cells (headarrow), vacuolization (V), dilatation (d), edema (e), X40, Bar: 20 µm.
(See color figure at http://tipbilimleri.turkiyeklinikleri.com/)

sulin was homogeneous whilst that of glucagon was denser towards the islet margins (Figures 5 and 6).

In the sections of the *Group K80*, some capillaries were dilated while some were constricted. Capillary endothelial swelling was similar to that of *group K40*. The zymogen granules in semi-thin sections showed degeneration and random distribution (Figure 3). The degeneration and vacuolation of the acinar cells were more intense than *group K60* (Figure 4). The intensity and distribution of staining for insulin and glucagon was similar to *group K 60* (Table 1), (Figures 5 and 6).

In *group K100*, the capillaries had luminal irregularity, more dilation, and more vacuolization compared to the other groups. There was also swelling of the endothelial cells in some areas of the capillaries (Figure 4). Staining for glucagon was strongest in this group (+++) whilst for insulin, the strength was the same as that of *group K40* (Table 1). Insulin and glucagon staining of the islets were homogeneous (Figure 5, 6).

Count of zymogen granules in K60 and K80 was statistically significantly higher compared to the control group ($p<0.005$). Acinar cell degeneration in K100 was statistically significantly higher compared to thecontrol group ($p<0.005$). Capillary lumen dilatation, vacuolization and endothelial swelling were statistically significantly more frequent in K60, K80 and K100 groups compared to K40 and the control groups ($p<0.005$). Immunopositivity for insulin and glucagon was statistically significantly higher in the control group compared to the study groups ($p<0.005$), but did not differ between the study groups. ($p>0.005$).

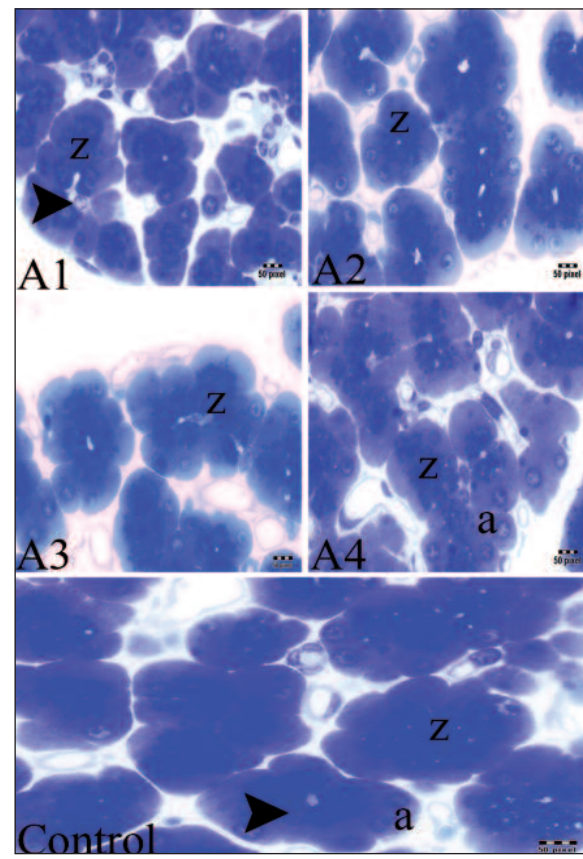

FIGURE 4: A1, B1, C1, D1 (group 1), A2, B2, C2, D2 (group 2), A3, B3, C3, D3 (group 3), A4, B4, C4, D4 (group 4) and Control group, A1, A2, A3, A4 and Control: Micrographs of semi-thin sections stained with toluidine blue in pancreas. Zymogene granules (Z), acinus, acinar cell (a), centroacinar cells (headarrow), X40, Bar: 50 pixel.
(See color figure at http://tipbilimleri.turkiyeklinikleri.com/)

TABLE 1: The results of pathological and immunohistological findings, evaluated by a three point scoring system.

Groups	Zymogen granules			Acinar cell degeneration			Capillary lumen dilatation			Capillary vacuolization			Capillary endothelial swelling			Immunopositivity for insulin			Immunopositivity for glucagon		
	Median	Min-Max	Score	Median	Min-Max	Score	Median	Min-Max	Score	Median	Min-Max	Score	Median	Min-Max	Score	Median	Min-Max	Score	Median	Min-Max	Score
Control Group	1.00	1.00-2.00	+	1.00	1.00-2.00	+	1.00	1.00-2.00	+	0.00	0.00	-	0.00	0.00	-	3.00	2.00-4.00	+++	3.00	3.00	+++
K40	1.00	1.00-2.00	+	1.00	1.00-2.00	+	2.00	1.00-3.00	++	0.00	0.00	-	0.00	0.00	-	2.00	1.00-3.00	++[d]	1.00	1.00-2.00	+[d]
K60	3.00	2.00-4.00	+++[a]	2.00	1.00-3.00	++	3.00	2.00-4.00	+++[c]	2.00	1.00-2.00	++[c]	2.00	1.00-2.00	++[c]	2.00	2.00	++[d]	1.00	1.00	+[d]
K80	3.00	3.00-4.00	+++[a]	2.00	1.00-2.00	++	3.00	2.00-4.00	+++[c]	2.00	2.00-3.00	++[c]	2.00	2.00-3.00	++[c]	1.00	1.00	+[d]	1.00	1.00-2.00	+[d]
K100	1.00	1.00	+	3.00	2.00-4.00	+++[b]	3.00	2.00-4.00	+++[c]	3.00	3.00-4.00	++[c]	3.00	3.00-4.00	++[c]	1.00	1.00	+[d]	1.00	1.00-2.00	+[d]

[a]: K60 and K80 compared with the control group ($p < 0.005$).
[b]: K100 compared with the control group ($p < 0.005$).
[c]: K60, K80 and K100 compared with K40 and the control group ($p < 0.005$).
[d]: The study groups compared with the control group ($p < 0.005$).

FIGURE 5: C1, C2, C3, C4 and Control: Micrographs of insulin immunopositive Beta cells in endocrine islets of pancreas. Immunopositive cells (small arrows), X40, Bar: 20 µm.
(See color figure at http://tipbilimleri.turkiyeklinikleri.com/)

DISCUSSION

Our study showed histopathologic alterations associated with increased dosages of ketamine on light microscopy. Changes in K40 group were similar to the control group. However, significant histological changes were observed in the pancreatic tissue in all other groups. This study revealed that prolonged administration of ketamine doses higher than 40 mg/kg can cause pathological alterations visible under light microscopy. EM further confirmed and detailed the histopathological alterations in each study group.

It is well known that ketamine increases blood glucose levels. The acute hyperglycemic effect of ketamine and xylazine in part reflects α_2-adrenoceptor-dependent changes of gluco-regulatory hor-

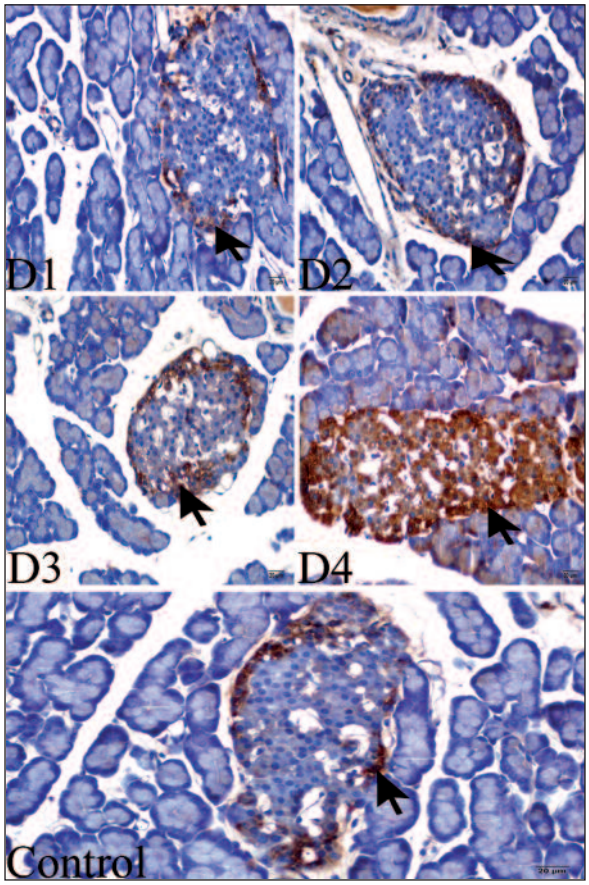

FIGURE 6: D1, D2, D3, D4 and Control: Micrographs of glucagon immunopositive Alpha cells in endocrine islets of pancreas. Immunopositive cells (small arrows), X40, Bar: 20 µm.
(See color figure at http://tipbilimleri.turkiyeklinikleri.com/)

mones such as insulin, corticosterone, growth hormone, and adrenocorticotropic hormone.[12] Therefore, it is very likely that the mode of action of ketamine involves pancreatic islet cells, and prolonged use of ketamine may cause functional and morphological alterations of the pancreatic tissue, similar to other tissues (heart and neurons) studied previously.[18,19]

Severe acute pancreatitis is characterized by pancreatic necrosis, resulting in local and systemic inflammation. Pancreatitis affects both the systemic and pancreatic vasculature.[20] In addition, disturbances in microcirculation play a significant role in pathogenesis of pancreatitis.[21] Experimental chronic pancreatitis is characterized by low pancreatic blood flow, low interstitial pH, and impaired pancreatic tissue oxygenation, which are all findings consistent with the ischaemia-reperfusion mechanisms.[22] Microcirculation of pancreas is disturbed due to conditions like dilatation of capillary lumen, capillary vacuolation and endotelial swelling. Our study showed that these findings were more frequent in rats receiving long-term ketamine infusion, suggesting that ketamine may disturb microcircultaion of pancreas. Acute pancreatitis may occur after changes in microvascular anatomy. Further studies are needed to confirm whether the disturbed microcirculation may cause pancreatitis or not.

Some studies involve myocardium and neural tissue. Long-term application of ketamine has been demonstrated to cause significant ventricular myocardial apoptosis, fibrosis and sympathetic sprout, which changed the electrophysiological properties of the heart and increased susceptibility to malignant arrhythmia that may lead to sudden cardiac death.[23] It has also been shown that neuronal Ca^{2+} oscillations mediate neuronal differentiation and synaptogenesis via activating the Ca^{2+}/calmodulin kinase II. By acting via the N-methyl-d-aspartate receptor, ketamine exerts its toxic effect through the suppression of neuronal Ca^{2+} oscillations, down-regulation of the Ca^{2+}/calmodulin kinase II, and consecutively reduces synaptic integrity.[19]

EM is an accepted method to depict the histopathological changes in the pancreatic tissue. The immunohistochemical staining of pancreatic acinar cells provides understanding of exocrine functions of those cells.[24] We have also included these well-accepted techniques in our current study.

There are several immune-histochemical and morphometric studies in the literature on glucagon, insulin, and somatostatin regarding their immunereactivity in the pancreatic cells.[25-28]

Our study showed no statistically significant difference between study groups in terms of secreted insulin or glucagon levels. In addition, we observed that ketamine caused a dose-dependent decrease in insulin and glucagon levels, suggesting that ketamine may inhibit secretory functions of pancreas.

There are number of limitations in current study. However, the main limitation of this study is the requirement of relatively high dose of sedatives in rats. Ketamine dose for sedation in rats is 75-100 mg/kg i.p. This dose is much higher than the sedative dose required in humans, mainly due to high metabolic rate of rats. Therefore, doses of 40-100 mg/kg were administered and investigated in this study. Human studies with clinically relevant doses are required to determine the effect of long-term ketamine in human pancreatic functions and structure. In current study, ketamine was given by i.p. injection, this route is not used in humans. However, IV injection in rats may interfere with the nutrition of the animals and is more difficult to apply than an i.p. injection, which is why the i.p. route is more often chosen. Furthermore, i.p. ketamine injection seems to cause less damage to rats when compared to IV.[29] Another and very important limitation of our study is that it does not provide any insight to clinical impact of these changes.

In conclusion, in rats, long-term ketamine use has pathological effects on pancreatic histology. Further research in the rat model is needed to demonstrate whether these alterations are permanent or reversible. The relevance of the animal model findings to humans will require clinical trials, but in the meantime, caution should be exercised on the amount and duration of ketamine administration.

REFERENCES

1. Patterson DR, Hofland HW, Espey K, Sharar S; Nursing Committee of the International Society for Burn Injuries. Pain management. Burns 2004;30(8):A10-5.
2. Trupkovic T, Kinn M, Kleinschmidt S. Analgesia and sedation in the intensive care of burn patients: results of a European survey. J Intensive Care Med 2011;26(6):397-407.
3. Stoddard FJ, Sheridan RL, Saxe GN, King BS, King BH, Chedekel DS, et al. Treatment of pain in acutely burned children. J Burn Care Rehabil 2002;23(2):135-56.
4. Visser E, Schug SA. The role of ketamine in pain management. Biomed Pharmacother 2006;60(7):341-8.
5. Dahan A, Olofsen E, Sigtermans M, Noppers I, Niesters M, Aarts L, et al. Population pharmacokinetic-pharmacodynamic modeling of ketamine-induced pain relief of chronic pain. Eur J Pain 2011;15(3):258-67.
6. Goldberg ME, Domsky R, Scaringe D, Hirsh R, Dotson J, Sharaf I, et al. Multi-day low dose ketamine infusion for the treatment of complex regional pain syndrome. Pain Physician 2005;8(2):175-9.
7. Krupitsky EM, Grinenko AY. Ketamine psychedelic therapy (KPT): a review of the results of ten years of research. J Psychoactive Drugs 1997;29(2):165-83.
8. Krystal JH, Madonick S, Perry E, Gueorguieva R, Brush L, Wray Y, et al. Potentiation of low dose ketamine effects by naltrexone: potential implications for the pharmacotherapy of alcoholism. Neuropsychopharmacology 2006; 31(8):1793-800.
9. Sørensen AG, Barnung S, Rasmussen LS. [Ketamine is used again by both physicians and addicts]. Ugeskr Laeger 2011;173(35): 2123-6.
10. Morgan CJ, Curran HV; Independent Scientific Committee on Drugs. Ketamine use: a review. Addiction 2012;107(1):27-38. doi: 10.1111/j.1360-0443.2011.03576.x.
11. Rodrigues SF, de Oliveira MA, Martins JO, Sannomiya P, de Cássia Tostes R, Nigro D, et al. Differential effects of chloral hydrate- and ketamine/xylazine-induced anesthesia by the s.c. route. Life Sci 2006;79(17):1630-7.
12. Saha JK, Xia J, Grondin JM, Engle SK, Jakubowski JA. Acute hyperglycemia induced b y ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. Exp Biol Med (Maywood) 2005;230(10):777-84.
13. Reyes Toso CF, Linares LM, Rodríguez RR. Blood sugar concentrations during ketamine or pentobarbitone anesthesia in rats with or without alpha and beta adrenergic blockade. Medicina (B Aires) 1995;55(4):311-6.
14. Sear JW. Ketamine hepato-toxicity in chronic pain management: another example of unexpected toxicity or a predicted result from previous clinical and pre-clinical data? Pain 2011;152(9):1946-7.
15. Hindlycke M, Jansson L. Glucose tolerance and pancreatic islet blood flow in rats after intraperitoneal administration of different anesthetic drugs. Ups J Med Sci 1992;97(1):27-35.
16. Rodacker M, Jin L, Zhang S, Lloyd RV. Insulin and Glucagon mRNA Expression and Prohormone Convertase Immunoreactivity in Normal and Neoplastic Pancreatic Endocrine Tissue. Endocr Pathol 2000;11(2):179-183.
17. Bancroft JD, Cook HC. Manual of Histological Techniques. 1st ed. London: Churchill Livingstone Medical Division Longman Group Limited; 1984.p.ii-274.
18. Li Y, Shi J, Yang BF, Liu L, Han CL, Li WM, et al. Ketamine-induced ventricular structural, sympathetic and electrophysiological remodelling: pathological consequences and protective effects of metoprolol. Br J Pharmacol 2012;165(6):1748-56.
19. Sinner B, Friedrich O, Zink W, Zausig Y, Graf BM. The toxic effects of s(+)-ketamine on differentiating neurons in vitro as a consequence of suppressed neuronal Ca2+ oscillations. Anesth Analg 2011;113(5):1161-9.
20. Cuthbertson CM, Christophi C. Disturbances of the microcirculation in acute pancreatitis. Br J Surg 2006;93(5):518-30.
21. Woeste G, Wullstein C, Meyer S, Usadel KH, Hopt UT, Bechstein WO, et al. Octreotide attenuates impaired microcirculation in postischemic pancreatitis when administered before induction of ischemia. Transplantation 2008;86(7):961-7.
22. Toyama MT, Lewis MP, Kusske AM, Reber PU, Ashley SW, Reber HA. Ischaemia-reperfusion mechanisms in acute pancreatitis. Scand J Gastroenterol Suppl 1996;219:20-3.

23. Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 1989;244(4910):1360-2.

24. Momose K, Nunomiya S, Nakata M, Yada T, Kikuchi M, Yashiro T. Immunohistochemical and electron-microscopic observation of beta-cells in pancreatic islets of spontaneously diabetic Goto-Kakizaki rats. Med Mol Morphol 2006;39(3):146-53.

25. Adewole SO, Ojewole JA. Insulin-induced immunohistochemical and morphological changes in pancreatic beta-cells of streptozotocin-treated diabetic rats. Methods Find Exp Clin Pharmacol 2007;29(7):447-55.

26. Fu Q, Honda M, Ohgawara H, Igarashi N, Toyada C, Omori Y, et al. Morphological analysis of pancreatic endocrine cells in newborn animals delivered by experimental diabetic rats. Diabetes Res Clin Pract 1996;31(1-3):57-62.

27. Gülmez N, Kocamış H, Aslan Ş, Nazlı M. Immunohistochemical distribution of cells containing insulin, glucagon and somatostatin in the goose (Anser anser) pancreas. Turk J Vet Anim Sci 2004;28(2):403-7.

28. Badawoud MH. Immunohistochemical and morphometric study of the effect of maternal diabetes on rat foetal pancreatic islets. Folia Morphol (Warsz) 2003;62(1):19-24.

29. Smiler KL, Stein S, Hrapkiewicz KL, Hiben JR. Tissue response to intramuscular and intraperitoneal injections of ketamine and xylazine in rats. Lab Anim Sci 1990;40(1):60-4.