Determination of Vessel Wall Injury Induced by Intra-Arterial Midazolam in Rats and Histopathological Evaluation of 1.5 mg or 3 mg Lidocaine and Papaverine Treatment

Sıçanlarda Midazolamın İntraarteriyel Uygulaması Sonucunda Damar Duvarında Oluşan Zedelenmenin Belirlenmesi ve 1.5 mg ya da 3 mg Lidokain ve Papaverin ile Tedavisinin Histopatolojik Olarak Değerlendirilmesi

Aslı AYAZ,[a]
Nalan ÇELEBİ,[b]
Ersin Gürkan DUMLU,[c]
Dilara ZEYBEK,[d]
Altan ŞAHİN,[b]
Ülkü AYPAR[b]

[a]Clinic of Anaesthesiology,
Turkish Religious Foundation,
29 Mayıs Private Hospital,
Departments of
[b]Anaesthesiology,
[d]Histology and Embriology,
Hacettepe University Faculty of Medicine,
[c]Clinic of General Surgery,
Atatürk Research and Training Hospital,
Ankara

Geliş Tarihi/*Received:* 14.06.2013
Kabul Tarihi/*Accepted:* 13.12.2013

Yazışma Adresi/*Correspondence:*
Ersin Gürkan DUMLU
Atatürk Research and Training Hospital,
Clinicof General Surgery, Ankara,
TÜRKİYE/TURKEY
gurkandumlu@gmail.com

ABSTRACT Objective: We aimed to evaluate the histopathological changes of intra-arterial midazolam injection on the arterial wall in a rat model and the effectiveness of increasing doses of lidocaine, a local anaesthetic, and papaverin, a peripheric vasodilator, on midazolam-induced arterial damage. **Material and Methods:** In 72 adult Wistar albino rats, a linear abdominal incision was performed under anesthesia and, midazolam with or without 1.5 mg or 3 mg lidocaine or papaverine was injected under the branching area of the renal artery. The abdominal aorta section, which was distal to the injected area, was resected 1, 15 and 30 min after injection. The study consisted of a control group, which received no injection, and five main study groups and 11 subgroups, which differed by lidocaine and papaverine doses and time of evaluation. Samples were evaluated under transmission electron microscopy. Endothelial damage, interstitial oedema, lamellar damage, and neutrophil/lymphocyte infiltration were scored from 0 (non-damaged) to 4 (severe damage). **Results:** Midazolam induced damage in arterial wall that was greatest at 30 min post-injection. Damage was less severe in the papaverin-injected group than lidocaine-injected subjects. The endothelial damage, lamella damage and interstitial oedema were less severe at the high lidocaine dose (3 mg) than low lidocaine dose (1.5 mg). **Conclusion:** Midazolam injection into artery induces endothelial damage, lamella damage, interstitial oedema and neutrophil infiltration that were increased with time. Blood vessel wall damage caused by intra-arterial injection of midazolam can be prevented by papaverine or lidocaine in early stages. Therefore intra-arterial injection of midazolam should be treated promptly for long-term application of drugs.

Key Words: Midazolam; papaverine; lidocaine; injections, intra-arterial

ÖZET Amaç: Çalışmamızın amacı midazolamın intraarteriyel enjeksiyon hasarını tespit etmek ve bir lokal anestezik ilaç olan lidokain ve vazodilatatör etkili papaverinin tedaviye erken yanıtta etkilerinin doz artışına yanıtı ile birlikte incelenmesidir. **Gereç ve Yöntemler:** Toplam 72 yetişkin Wistar albino sıçanında, genel anestezi altında abdominal insizyon gerçekleştirildi ve 1.5 mg ya da 3 mg lidokain veya papaverin varlığında ya da yokluğunda midazolam renal arter dallanma alanının altında enjekte edildi. Enjeksiyon alanının distalindeki abdominal aorta enjeksiyondan 1, 15 ve 30 dak sonra çıkarıldı. Çalışma, enjeksiyon uygulanmayan kontrol grubu ve lidokain ve papaverin dozu ve değerlendirme zamanına göre değişen 5 ana ve 11 alt gruptan oluşuyordu. Örnekler transmision elektron mikroskobu ile değerlendirildi. Endotelyal hasar, interstisiyel ödem, lamellar hasar ve nötrofil/lenfosit infiltrasyonu 0 (hasarsız) ve 4 (ileri hasar) arasında skorlandı. **Bulgular:** Midazolam arteriyel duvarda enjeksiyon sonrası en fazla 30. dakikada olmak üzere hasar oluşturdu. Hasar papaverin uygulanan grupta lidokain grubuna göre daha azdı. Endotelyal hasar, interstisiyel ödem, lamellar hasar yüksek lidokain dozunda (3 mg), düşük lidokain dozuna (1,5 mg) göre daha azdı. **Sonuç:** Midazolamın artere enjeksiyonu zaman içinde artan endotelyal hasar, interstisiyel ödem, lamellar hasar ve nötrofil infiltrasyonu oluşturur. Damar duvarındaki hasar erken evrede papaverin ya da lidokain uygulanması ile azaltılabilir. Bu nedenle intraarteriyel midazolam enjeksiyonu sırasında bu ilaçlarla tedaviye acil olarak başlanmalı ve uzun süre devam edilmelidir.

Anahtar Kelimeler: Midazolam; papaverin; lidokain; intraarteryel enjeksiyonlar

Turkiye Klinikleri J Anest Reanim 2013;11(3):109-20

Accidental intra-arterial injection is a common occurrence, especially during anaesthetic and intensive care procedures. There are several case reports on this matter involving medical staff and substance users.[1-4] Intra-arterial injection can lead to medical complications that can result in limb amputation related to tissue necrosis.[5] Therefore, beginning proper treatment after an intra-arterial injection is very important. All medical staff practicing intravenous sedation on a routine basis must be informed about the symptoms and the treatment of accidental intra-arterial drug injection.[6,7]

Rapid ischemia is generally induced by intra-arterial drug injection. Various mechanisms can contribute to rapid ischemia, including crystal formation of the drug in the veins, hemolysis and platelet aggregation secondary to intimal damage, and venous construction and direct cytotoxicity causing stasis and thrombosis.[8,9]

Different treatment approaches were used against ischemia. Khan et al. reported an accidental intra-arterial injection of thiopental on the dorsum of the foot which was treated successfully with lidocaine and heparin together with leg rising, preventing a gangrenous episode of the extremity.[10] Bittner et al. treated accidental intra-arterial injection into brachial artery with intra-arterial urokinase and papaverine along with systemic heparinization and axillary plexus anesthesia with bupivacaine.[11] Both study emphasized the importance of timely and prompt treatment.

Midazolam, is a substance frequently used in operating rooms and intensive care units for sedation.[12,13] Its effects on the blood vessel wall during accidental intra-arterial injections have not been studied extensively.

In this study, we aimed to determine the histopathological changes of intra-arterial midazolam injection on the arterial wall in a rat model and to evaluate the effectiveness of increasing doses of lidocaine, a local anaesthetic, and papaverin, a peripheric vasodilator, on midazolam-induced arterial damage.

MATERIAL AND METHODS

STUDY DESIGN

This was an experimental animal study. Seventy-two adult Wistar albino rats weighing 250-300 g that were raised under identical conditions at the Hacettepe University Faculty of Medicine Experimental Animals Laboratory were used. This study was approved by the Hacettepe University Ethics Committee for Animal Experiments (Date: 08.01.2008, Dossier Registry No: 2007, Decision No: 60). All animals that were involved in experiments have received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html).

OPERATIONAL PROCEDURES

Rats were anesthetised with an intramuscular (i.m.) injection of 80 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, İstanbul, Turkey) and 2% xylazine hydrochloride (Rompun, Bayer, İstanbul, Turkey) in 10 mg/kg i.m. doses. Following anesthesia, the incision area was properly sanitised. A linear incision was performed through the abdominal skin and muscle layers from the xiphoid process parallel to the costal margin. Once the abdominal aorta was reached, the injection procedures were performed under the branching area of the renal artery (Figure 1a). The abdominal aorta section, which was distal to the injected area, was resected (Figure 1b). Tissue samples were taken 1, 15 and 30 min after injection, and the rats were then sacrificed by cervical dislocation. Vital signs were not monitored due to short duration of the experiment.

The resected segments were placed in 2% glutaraldehyde solution and sent for histological examination.

STUDY GROUPS

The study consisted of a control group (n=6), which received no intra-arterial injections, and five main study groups and 11 subgroups, which differed by lidocaine and papaverine doses and time of evaluation as follows:

Group 1 (midazolam): 5 mg/kg midazolam was applied through the abdominal aorta. Group 1 was

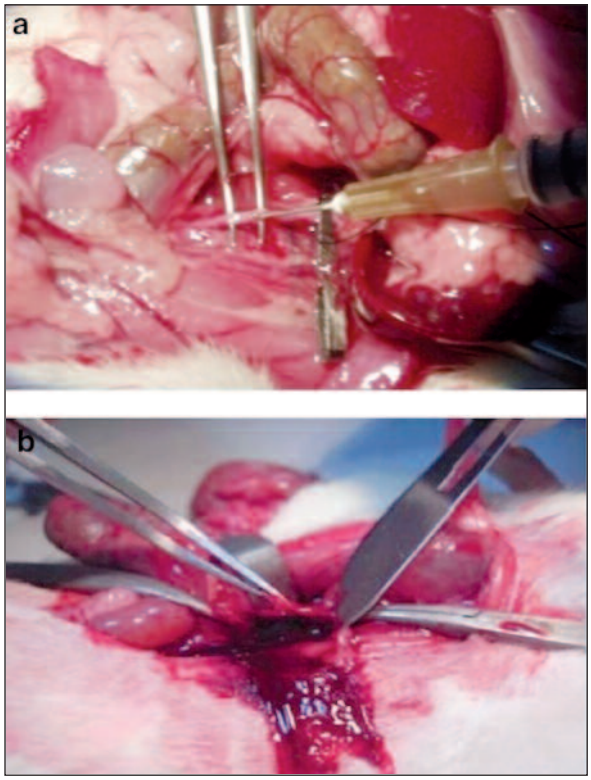

FIGURE 1: (a) Injection through abdominal aorta under the branching area of the renal artery. **(b)** The abdominal aorta section, which was distal to the injected area, was resected.
(See color figure at
http://www.turkiyeklinikleri.com/journal/anesteziyoloji-reanimasyon-dergisi/1304-0499/)

divided into three subgroups (n=6 in each). Samples were collected 1, 5 and 30 min post-injection in Groups 1a, 1b and 1c, respectively.

Group 2 (midazolam+lidocaine 1.5 mg): 1.5 mg/kg of lidocaine was injected following injection of 5 mg/kg of midazolam through the abdominal aorta. Group 2 was divided into two subgroups (n=6 in each). Samples were collected 15 and 30 min after lidocaine injection in Groups 2a and 2b, respectively.

Group 3 (midazolam+papaverine 1.5 mg): Following injection of 5 mg/kg midazolam, 1.5 mg/kg papaverine was injected through the abdominal aorta. Group 3 was divided into two subgroups (n=6 in each). Samples were collected 15 and 30 min post-injection in Groups 3a and 3b, respectively.

Group 4 (midazolam+lidocaine 3 mg): Following the application of 5 mg/kg midazolam, 3 mg/kg lidocaine was injected through the abdominal aorta. Group 4 was divided into two subgroups (n=6 in each). Samples were collected 15 and 30 min post-injection in Groups 4a and 4b, respectively.

Group 5 (midazolam+papaverine 3 mg): Following the application of 5 mg/kg midazolam, 3 mg/kg papaverine was injected through the abdominal aorta. Group 5 was divided into two subgroups (n=6 in each). Samples were collected 15 and 30 min post-injection in Groups 5a and 5b, respectively.

Lidocaine and papaverine doses were determined according to literature.[14,15]

The study groups were summarized in Table 1.

TRANSMISSION ELECTRON MICROSCOPY

Sample Preparation

Tissue samples were fixated in 2% glutaraldehyde at +4°C for two hours. A second fixation was

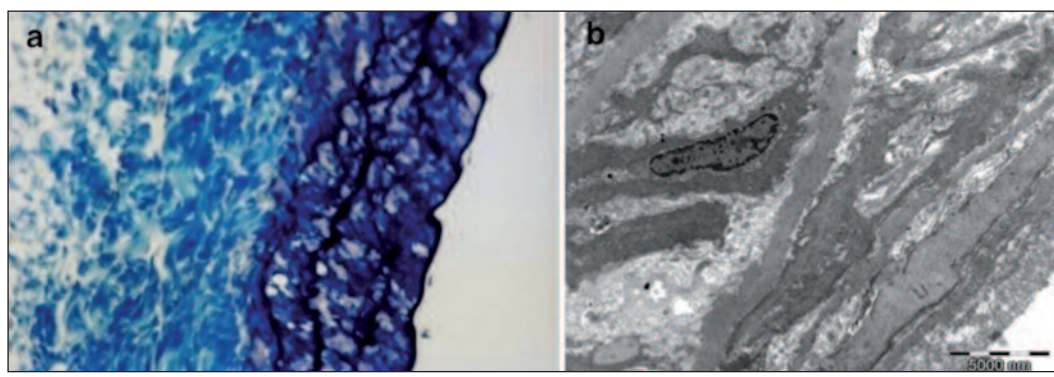

FIGURE 2: Images of control group. **(a)** The endothelial and subendothelial layers preserved their continuity (Methylene blue-Azur II, x400). **(b)** The over-lapping junctions were in good condition (Uranyl acetate-lead citrate, x5000).
(See color figure at http://www.turkiyeklinikleri.com/journal/anesteziyoloji-reanimasyon-dergisi/1304-0499/)

performed with 1% osmium tetroxide for 90 min. Tissues were monitored by routine electron microscope tissue processing by being changed and cleaned 3-4 times with phosphate buffered saline.

Electron Microscope Tissue Monitoring

The fixed tissue samples were placed into the electron microscopy processing device (Leica EM TP) for dehydration and infiltration. Half thin sections were painted with a mixture of 1% methylene blue-Azure II and 1% borax. Thick sections were examined and photographed with a Leica DM6000B (Wetzlar, Germany) microscope connected to a DFC490 digital camera (Leica, Wetzlar, Germany). Silver-coloured thin sections were rounded up in copper grids after being collected with a glass knife on a Leica Ultracut R microtome. Thin sections were painted with uranyl acetate and lead citrate on a grid painting device (Leica EM AC20, Wetzlar, Germany). The sections were examined and photographed by a JEOL-1400 electron microscope and Gatan Oriun SC1000 CCD camera.

HISTOLOGICAL PARAMETERS

The following histological parameters were used to define the damage: endothelial damage, interstitial oedema, lamellar damage, and neutrophil/lymphocyte infiltration. Damage was scored as follows: 0 for non-damaged subjects, 1 for very light damage, 2 for light damage, 3 for moderate damage and 4 for severe damage[16].

STATISTICAL ANALYSIS

The number of rats that perished was kept to a minimum. The statistical power (β) of the study with 72 animals was calculated as 0.92 with an α error of 5%.

The study data were summarized using descriptive statistics (mean±standard deviation for quantitative data; frequency and percentage for qualitative data). The study groups were compared with the Mann Whitney U test for quantitative data.

A value of $p<0.05$ was considered statistically significant. Statistical analysis was executed using a commercially available software (Statistical Package for Social Sciences, version 13.0, SPSS Inc., Chicago, Illinois, USA).

RESULTS

In the control group, the tunica intima, tunica adventitia, and tunica media of the aortic wall, which were assessed individually, had normal histopathology. The organelle distribution of the endothelial cells and the chromatin distribution in the nuclear ultrastructure were regular under electron microscopy (Figure 2).

HISTOPATHOLOGY OF THE MIDAZOLAM GROUP

In Group 1a, although endothelial continuity in the aorta was maintained, there were several protrusions towards the lumen in relation to the intimal edema. Under electron microscopy, the cytoplasm and nuclei of the endothelial cells were normal (Figure 3a and 3b). In Group 1b, endothelial continuity in the aorta was also preserved. Compared with Group 1a, there were no differences in endothelial cells, elastic lamella or smooth muscle cells by electron microscopy (Figure 3c and 3d). In Group 1c, although the endothelial continuity and intimal edema in the aorta were diminished, there were several intimal areas in which there were protrusions towards the lumen, which was caused by infiltrative cells. Electron microscopy observations showed that endothelial cells and their indented nuclei and pynocytotic vesicles were regular (Figure 3e and 3f).

HISTOPATHOLOGY OF THE MIDAZOLAM+1.5 MG LIDOCAINE GROUP

In Group 2a, endothelial damage on the aorta was minimal. In some instances, the endothelial continuity was distorted and there was thrombus formation in the area. The electron microscopy evaluation revealed damage to the endothelial cells (Figure 4a and 4b). In Group 2b, intimal and endothelial continuity in the aorta was normal. Electron microscopy observations showed degeneration of endothelial and smooth muscle cell (Figure 4c and 4d).

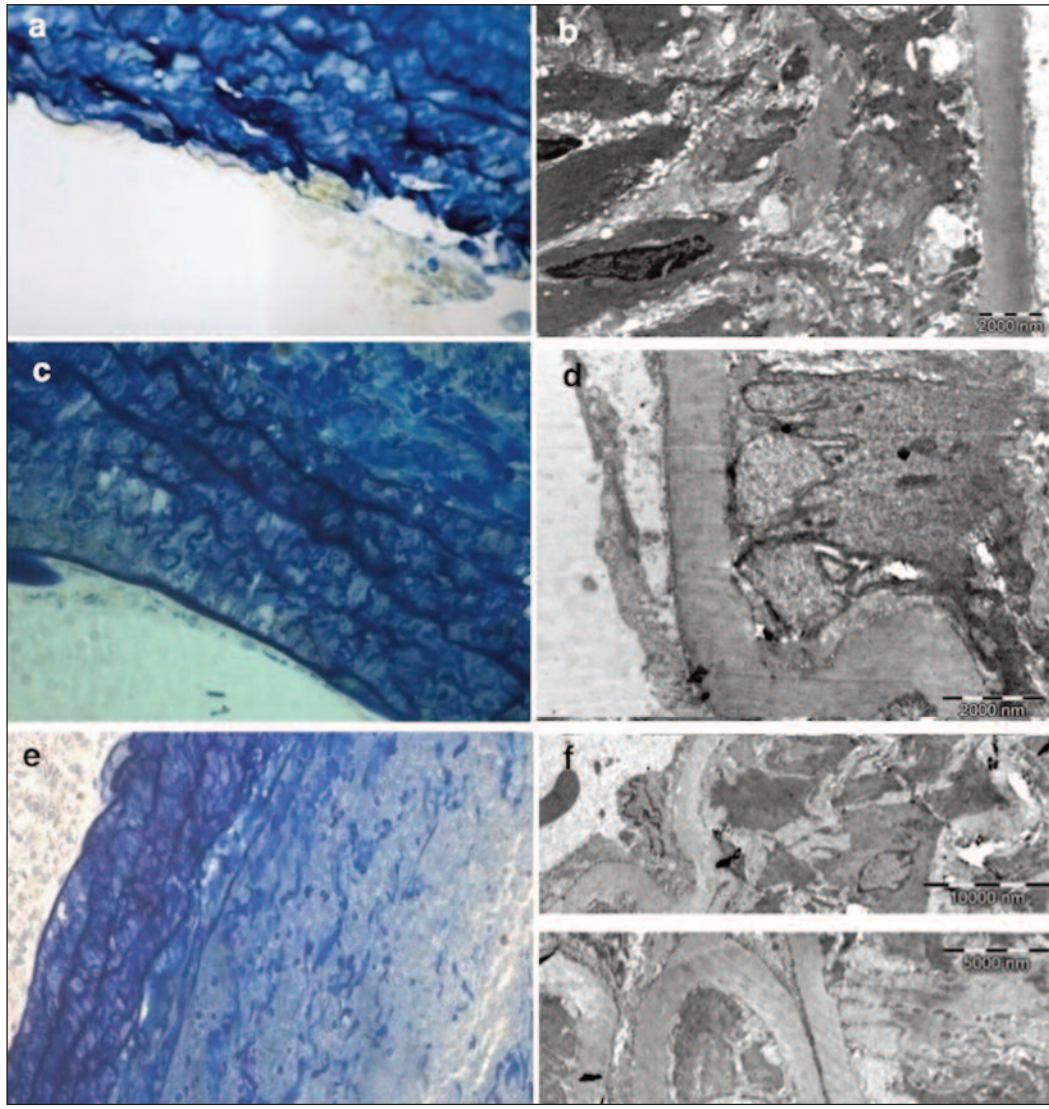

FIGURE 3: Images of Group 1. **(a)** Histopathology of aortic wall resected 1 min after midazolam shows that there were several protrusions towards the lumen in relation to the intimal oedema. (Methylene blue-Azur II, x400). **(b)** Electronmicrograph of aortic wall resected 1 min after midazolam shows that the cells were not separated from the basal lamina. (Uranyl acetate-lead citrate, x7500). **(c)** Histopathology of aortic wall resected 5 min after midazolam shows there was no apparent oedema in most areas. (Methylene blue-Azur II, x1000). **(d)** Electronmicrograph of aortic wall resected 5 min after midazolam shows endothelial cells, elastic lamella, and smooth muscle cells (Uranyl acetate-lead citrate, x12000). **(e)** Histopathology of aortic wall resected 30 min after midazolam shows that oedema on the tunica media was quite low. (Methylene blue-Azur II, x1000). **(f)** Electronmicrograph of aortic wall resected 30 min after midazolam shows that no dehiscence or divergence was observed. (Uranyl acetate-lead citrate).
(See color figure at http://www.turkiyeklinikleri.com/journal/anesteziyoloji-reanimasyon-dergisi/1304-0499/)

HISTOPATHOLOGY OF THE MIDAZOLAM + 1.5 MG PAPAVERINE GROUP

In Group 3a, blood vessel walls in the aorta were mostly preserved compared to the other groups. Electron microscopy observations showed that the nuclei of the endothelial cells spread over the lumen were long and flat and that the cytoplasm was quite thin (Figure 5a and 5b). In Group 3b, endothelial cells forming the intima in the aorta were normal. However, there was significant localised intimal thickening. Electron microscopy observations showed that endothelial cells diverged from the basal lamina in several areas and that there was dehiscence among smooth muscle cells and dense collagen fibrils on the adventitia (Figure 5c and 5d).

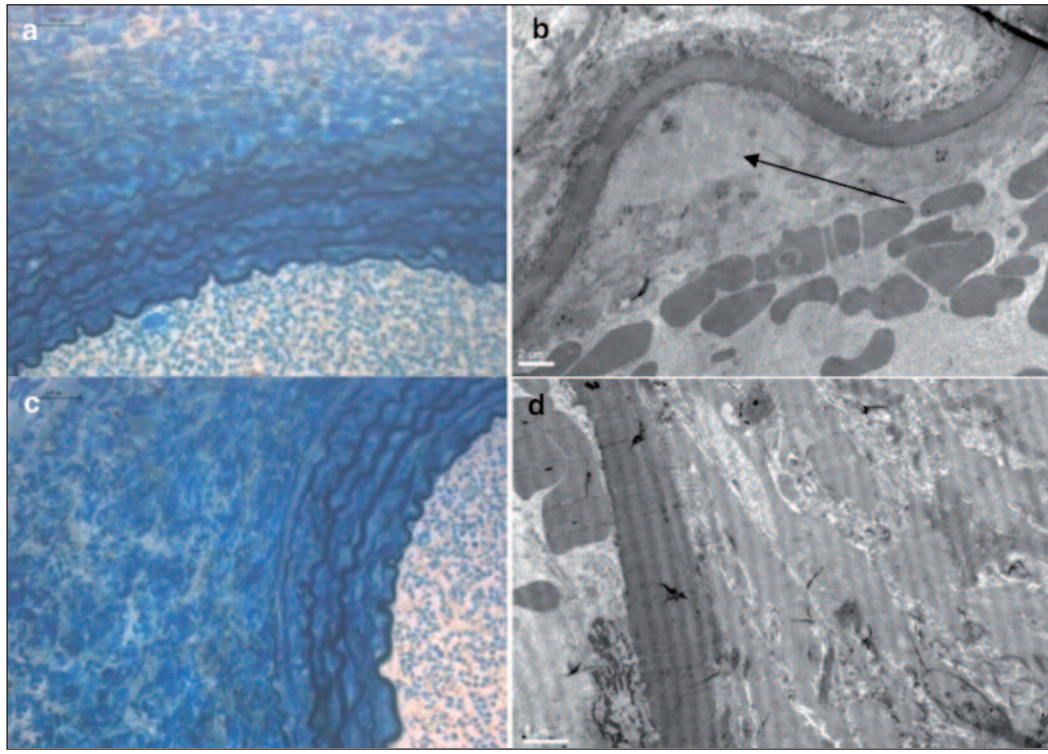

FIGURE 4: Images of Group 2. **(a)** Histopathology of aortic wall resected 15 min after midazolam+1.5 mg lidocaine shows that the endothelial continuity was distorted. (Methylene blue-Azur II). **(b)** Electronmicrograph of aortic wall resected 15 min after midazolam+1.5 mg lidocaine shows that there was intimal thickening in some areas (arrow). (Uranyl acetate-lead citrate). **(c)** Histopathology of aortic wall resected 30 min after midazolam+1.5 mg lidocaine shows that there was minimal damage to elastic lamella, (Methylene blue-Azur II). **(d)** Electronmicrograph of aortic wall resected 30 min after midazolam+1.5 mg lidocaine shows degeneration of endothelial cells. (Uranyl acetate-lead citrate).
(See color figure at http://www.turkiyeklinikleri.com/journal/anesteziyoloji-reanimasyon-dergisi/1304-0499/)

HISTOPATHOLOGY OF THE MIDAZOLAM+3 MG LIDOCAINE GROUP

In Group 4a, there was minimal damage to the continuity of the endothelial cells forming the intima in the aorta. Electron microscopy showed that endothelial cells were progressing towards apoptosis (Figure 6a and 6b). In Group 4b, endothelial continuity in the aorta was preserved. There was minimal damage to the elastic lamella forming the tunica media, as well as oedema-related dehiscences between lamella. Electron microscopy observations showed that endothelial continuity was preserved (Figure 6c and 6d).

HISTOPATHOLOGY OF THE MIDAZOLAM+3 MG PAPAVERINE GROUP

In Group 5a, there was damage to the endothelial region forming the tunica intima and to the elastic lamella forming the tunica media. Degeneration in endothelial cells and divergence in the basal lamina of the blood vessel wall were observed during electron microscopic processing (Figure 7a and 7b). In Group 5b, endothelial cells forming the intima were normal in some samples, whereas in other samples, there was minimal endothelial damage. Electron microscopy observations showed that endothelial continuity was maintained. The elastic lamella were parallel, undulant and regular (Figure 7c and 7d).

COMPARISONS OF DAMAGE SCORES BETWEEN GROUPS

When we compare the interstitial oedema and neutrophil infiltration parameters in midazolam-treated subjects, the extent of damage was significantly higher in 5 and 30 min post-injection groups compared to 1 min group ($p<0.05$ and $p<0.05$, respectively). Neutrophil infiltration in the 30 min group was significantly higher than in the 1 and 15 min groups; there were no differences

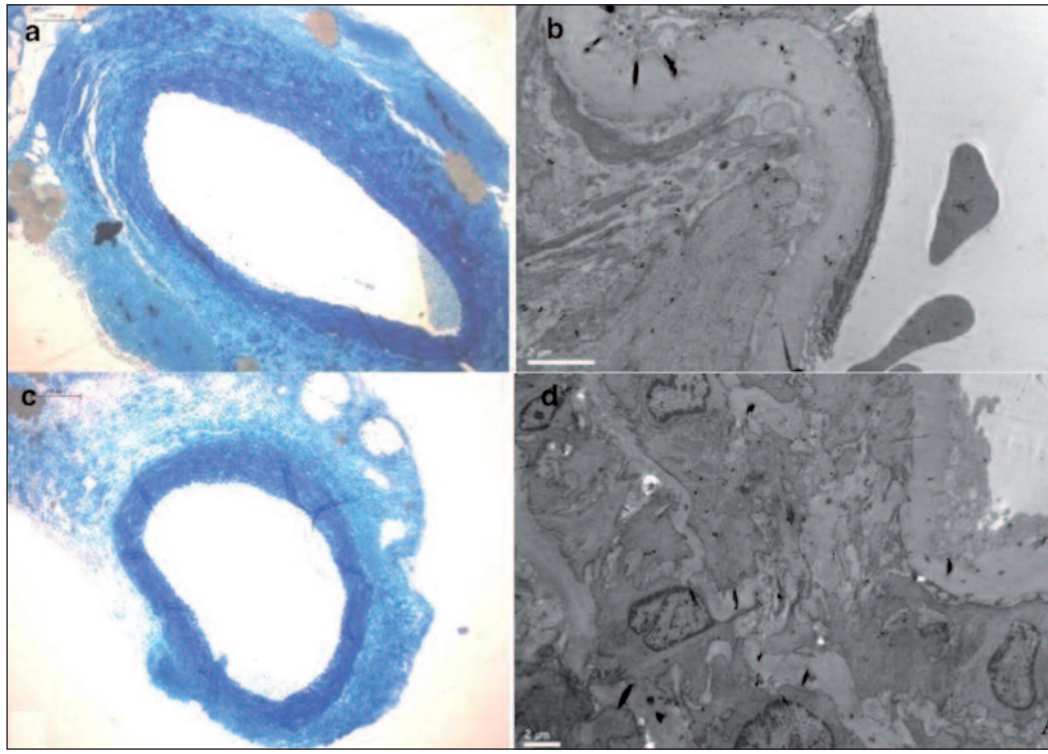

FIGURE 5: Images of Group 3. **(a)** Histopathology of aortic wall resected 15 min after midazolam+1.5 mg papaverine shows that blood vessel walls in the aorta were mostly preserved compared to the other groups.(Methylene blue-Azur II). **(b)** Electronmicrograph of aortic wall resected 15 min after midazolam+1.5 mg papaverine shows that the endothelial cells were damaged. (Uranyl acetate-lead citrate). **(c)** Histopathology of aortic wall resected 30 min after midazolam+1.5 mg papaverine shows that endothelial cells forming the intima in the aorta were normal. (Methylene blue-Azur II). **(d)** Electronmicrograph of aortic wall resected 30 min after midazolam+1.5 mg papaverine shows that endothelial cells diverged from the basal lamina in several areas (Uranyl acetate-lead citrate).
(See color figure at http://www.turkiyeklinikleri.com/journal/anesteziyoloji-reanimasyon-dergisi/1304-0499/)

between the 1 and 15 min groups ($p<0.05$, $p<0.05$, $p>0.05$, respectively). There was no difference between the three groups in terms of endothelial damage, lamella damage, or neutrophil/lymphocyte infiltration. The greatest midazolam-induced interstitial oedema and neutrophil infiltration was observed in subjects in which samples were collected 30 min post-injection.

When the effect of lidocaine and papaverine was compared, there was a significant difference between Group 2a and Group 3a in which samples were collected 15 min post-injection, with regard to endothelial damage and lamellar damage ($p=0.041$, $p=0.026$). Damage was less severe in the group injected with papaverine than in the group injected with lidocaine. There was no statistically significant difference between papaverine and lidocaine in preventing endothelial damage in the 30 min groups (Table 2).

The difference between Group 2a and Group 4a, which were injected with lidocaine, was statistically significant. Thus, endothelial damage ($p=0.041$), lamella damage ($p=0.015$) and interstitial oedema ($p=0.041$) were less severe at the higher lidocaine dose (3 mg) (Table 2). In terms of comparisons of 15 vs. 30 min groups, only the low-dose lidocaine (1.5 mg) showed a significant decrease in endothelial damage in the 30 min group compared to 15 min group ($p=0.041$) (Table 2).

DISCUSSION

In this experimental animal study, we found that midazolam injection into artery induces endothelial damage, lamella damage, interstitial oedema and neutrophil infiltration that were increased with time. Furthermore, blood vessel wall damage caused by intra-arterial injection of midazolam can be prevented by papaverine or

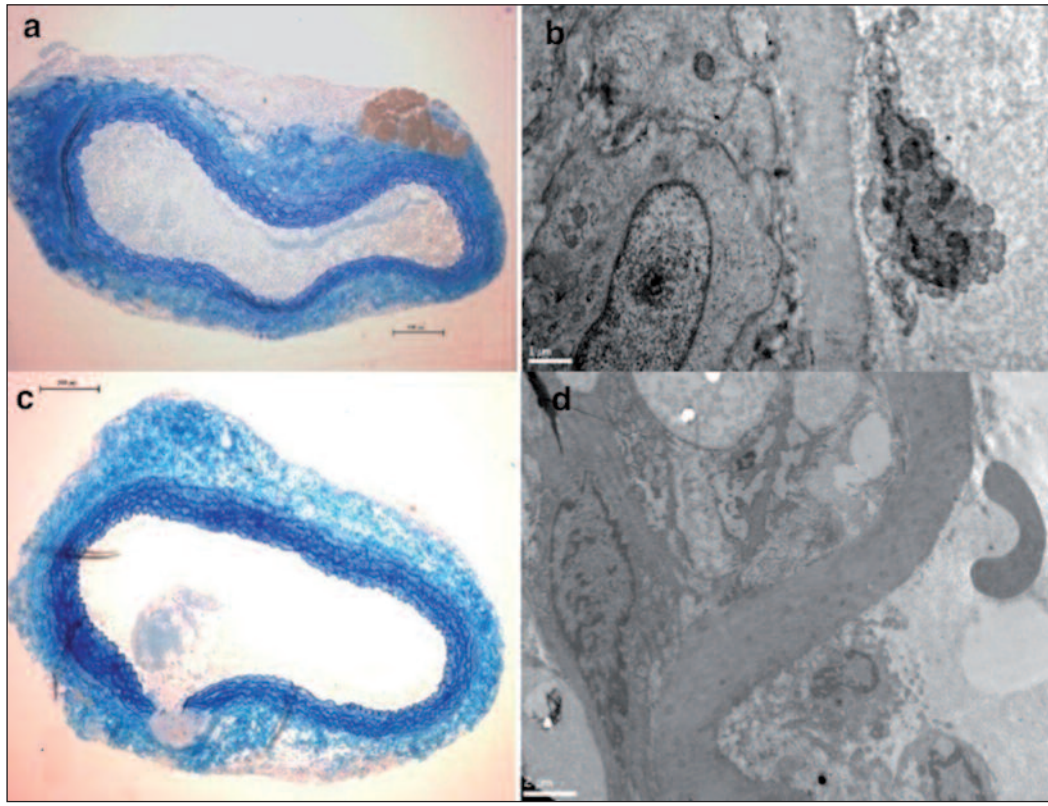

FIGURE 6: Images of Group 4. **(a)** Histopathology of aortic wall resected 15 min after midazolam+3 mg lidocaine shows that there was minimal damage to the continuity of the endothelial cells forming the intima in the aorta. (Methylene blue-Azur II). **(b)** Electronmicrograph of aortic wall resected 15 min after midazolam+3 mg lidocaine shows that endothelial cells were progressing towards apoptosis. (Uranyl acetate-lead citrate). **(c)** Histopathology of aortic wall resected 30 min after midazolam+3 mg lidocaine shows that endothelial continuity in the aorta was preserved. (Methylene blue-Azur II). **(d)** Electronmicrograph of aortic wall resected 30 min after midazolam+3 mg lidocaine shows that endothelial continuity was preserved. (Uranyl acetate-lead citrate).
(See color figure at http://www.turkiyeklinikleri.com/journal/anesteziyoloji-reanimasyon-dergisi/1304-0499/)

lidocaine in early stages. Therefore intra-arterial injection of midazolam should be treated promptly for long-term application of drugs.

Intra-arterial injection of intravenous drugs may result in local ischemia and apparent morbidities such as tissue necrosis[6,7]. Accidental intra-arterial drug injections can occur due to morbid obesity, the absence of contact with the patient during the injection, dark skin colour, and anatomic venous anomalies.[6,10] There are various complications in arterial cannulation. One of the greatest complications is ischemia, which can develop rapidly or late distal to the cannulation.[17] Other complications include local haematoma, local nerve damage, pseudoneurism, arteriovenous fistula, local celluloid and phlebitis. Systemic complications include sepsis, pulmonary thromboembolism, air embolism, catheter-related emboli and vasovagal syncope.

Accidental intra-arterial injection was first mentioned in the literature in 1943 in a case report presented by Macintosh and Heyworth involving thiopental. In this report, intra-arterial injection of thiopental at a concentration higher than 2% caused oedema, cyanosis, endothelial damage and necrosis, which progressed towards gangrene. The estimated rates for intra-arterial injection vary throughout the literature. Lundy[18] stated that the intra-arterial injection rate for thiopental was 1/8000, whereas Dundee[19] cited the rate for thiopental and other barbiturates as 1/3500, and Cohen[20] stated the rate as 1/56000 for intra-arterial drug injection.

Three disease case reports presented by Passie et al.[21] showed that following intra-arterial injection of pure diacetylmorphine among three substance abusers, vasospasm symptoms were

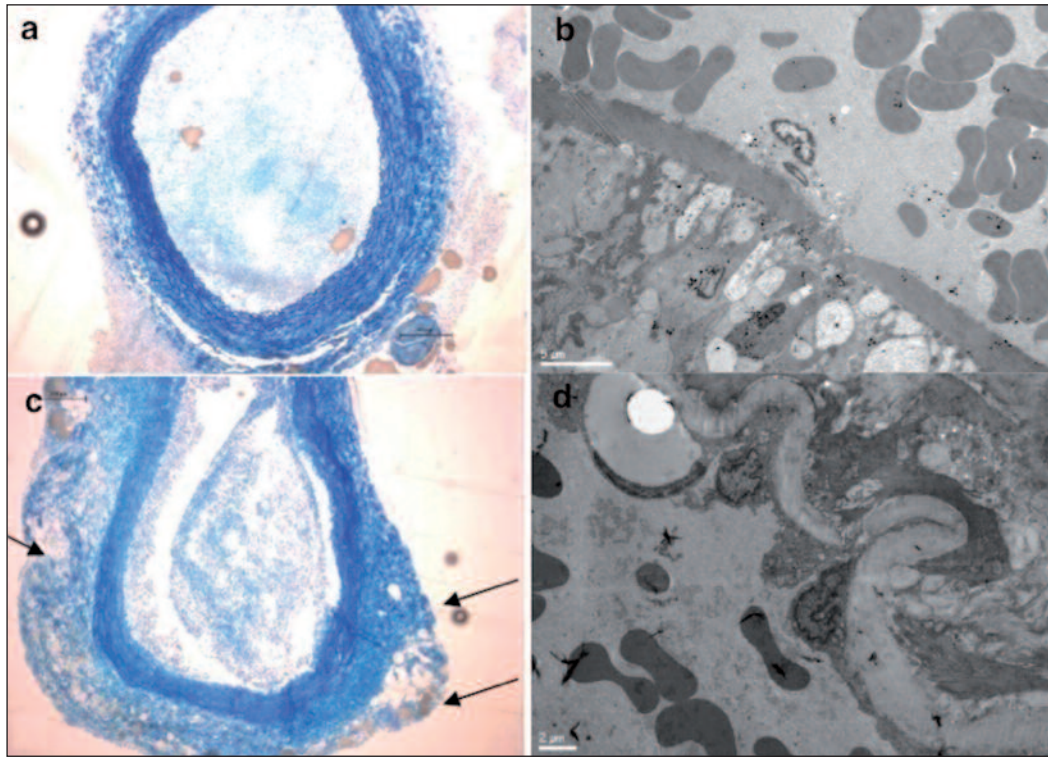

FIGURE 7: Images of Group 5. **(a)** Histopathology of aortic wall resected 15 min after midazolam+3 mg papaverine shows that there was damage to the endothelial region. (Methylene blue-Azur II). **(b)** Electronmicrograph of aortic wall resected 15 min after midazolam+3 mg papaverine shows degeneration in endothelial cells. (Uranyl acetate-lead citrate). **(c)** Histopathology of aortic wall resected 30 min after midazolam+3 mg papaverine shows adhesion on the endothelium. Neutrophilic infiltration and dehiscence between collagen fibrils were present on the tunica adventitia (arrows) (Methylene blue-Azur II). **(d)** Electronmicrograph of aortic wall resected 30 min after midazolam+3 mg papaverine shows that endothelial continuity was maintained. (Uranyl acetate-lead citrate).
(See color figure at http://www.turkiyeklinikleri.com/journal/anesteziyoloji-reanimasyon-dergisi/1304-0499/)

TABLE 1: Study groups (n=72).

Groups	Subgroups	Drug(s) injected intra-arterially	Post-injection time for resection of abdominal aorta section
Control group (n=6)		**None**	**Anytime**
Group 1 *(midazolam)* (n=18)	1a (n=6)	5 mg/kg midazolam	1 min
	1b (n=6)	5 mg/kg midazolam	5 min
	1c (n=6)	5 mg/kg midazolam	30 min
Group 2 *(midazolam+lidocaine 1.5 mg)* (n=12)	2a (n=6)	5 mg/kg midazolam 1.5 mg/kg of lidocaine	15 min
	2b (n=6)	5 mg/kg midazolam 1.5 mg/kg of lidocaine	30 min
Group 3 *(midazolam+papaverine 1.5 mg)* (n=12)	3a (n=6)	5 mg/kg midazolam 1.5 mg/kg of papaverin	15 min
	3b (n=6)	5 mg/kg midazolam 1.5 mg/kg of papaverin	30 min
Group 4 *(midazolam+lidocaine 3 mg)* (n=12)	4a (n=6)	5 mg/kg midazolam 3 mg/kg of lidocaine	15 min
	4b (n=6)	5 mg/kg midazolam 3 mg/kg of lidocaine	30 min
Group 5 *(midazolam+papaverine 3 mg)* (n=12)	5a (n=6)	5 mg/kg midazolam 3 mg/kg of papaverine	15 min
	5b (n=6)	5 mg/kg midazolam 3 mg/kg of papaverin	30 min

TABLE 2: Arterial wall damage scores of lidocaine- and papaverin-injected groups and p values for between groups comparisons (Mann Whitney U test).

	Study groups										p values					
	Control group	Group 1a (midazolam 5 mg) 1 min	Group 1b (midazolam 5 mg) 15 min	Group 1c (midazolam 5 mg) 30 min	Group 2a (lidocaine 1.5 mg) 15 min	Group 2b (lidocaine 1.5 mg) 30 min	Group 3a (papaverine 1.5 mg) 15 min	Group 3b (papaverine 1.5 mg) 30 min	Group 4a (lidocaine 3 mg) 15 min	Group 4b (lidocaine 3 mg) 30 min	Group 5a (papaverine 3 mg) 15 min	Group 5b (papaverine 3 mg) 30 min	Groups 2a vs. 3a	Groups 2b vs. 3b	Groups 2a vs. 4a	Groups 2a vs. 2b	Groups 3a vs. 5a	Groups 3b vs. 5b
Endothelial damage	0.00	0.60±0.12	0.70±0.18	0.70±0.22	1.00±0.63	0.16±0.40	0.16±0.40	0.00±0.00	0.16±0.40	0.14±0.32	0.60±0.30	0.66±0.81	0.041	0.699	0.041	0.041	0.12	0.180
Lamella damage	0.00	1.20±0.14	1.40±0.18	1.50±0.23	1.66±0.82	0.83±0.75	0.33±0.81	1.00±0.89	0.33±0.51	0.28±0.15	1.30±0.30	1.33±0.81	0.026	0.818	0.015	0.132	0.467	0.589
Interstitial oedema	0.00	1.70±0.35	2.00±0.46	2.3±0.65	2.50±0.84	2.16±1.17	3.00±0.00	3.00±0.63	1.33±0.81	1.30±0.80	3.00±0.50	3.00±0.89	0.394	0.240	0.041	0.699	0.872	1.000
Neutrophil/lymphocyte infiltration	0.00	1.70±0.54	2.10±0.62	2.60±0.74	2.33±0.82	2.00±0.63	3.00±0.00	2.50±0.54	2.00±0.00	1.80±0.30	2.60±1.00	2.66±1.03	0.180	0.240	0.394	0.485	0.600	0.699

Arterial wall damage scores were as follows: 0, no damage; 1, very light damage; 2, light damage; 3, moderate damage; 4, severe damage.
Data were presented as mean±standard deviation.

observed. In these cases, the patients recovered without any sequelae. Intra-arterial drug injections most commonly occur after uncontrolled drug use. Six hundred cases have been reported since 2002 in Germany. In a retrospective study, Trieman et al.[22] reviewed patients who experienced intra-arterial injection between 1977 and 1988. Among these, 48 patients were reported to experience limb ischemia. In a case report by Chong and Davis, following 5 mL intra-arterial injection of propofol, the patient felt intense pain on the distal side of the injection point, and paleness occurred on the forearm and on the palmar side of the hand.[23] After 30 min, pale areas started to blush, and after 4 hours, all causes of complaints had disappeared and no clinical sequelae were observed. The researchers emphasised that doctors should be careful of intra-arterial injections and that the patient's extremities can be preserved with rapid and appropriate treatments.

These studies clearly show that intra-arterial drug injections are too common to be ignored. The case reports on intra-arterial injections showed that patient complaints start at the time of injection, and clinical findings are clarified within 30 min to 1 hour. Therefore, the strategy we used of sampling at 1, 15, and 30 min post injection is consistent with the time intervals of symptomatic findings in case reports.

In a similar study conducted by Raininko, samples were collected at 5 and 20 min post injection, and effects were also evaluated after one hour.[24] We collected samples 1 min after injection because the symptoms of patients in intra-arterial injection cases were strongest at the time of injection. The fact that the symptoms were strong at the time of injection in case reports was a result of rapid changes to the blood vessel wall, which were caused by the drug. In cases in which amputation was performed due to necrosis distal to the injection, a study focused on peripheral muscular arteries rather than large elastic arteries may be useful. This study would require keeping the experimental animals alive for days, as such clinical complications take several days. However, we believe that rats are not ideal for such a study

because reaching the extremity arteries and performing the injection in rats is quite challenging. Thus, we examined the histopathological, rather than clinical, effects of intra-aortic injection.

During injections performed on patients, focusing on the patients' reaction and assessing them thoroughly is very important for the early diagnosis and treatment of intra-arterial injections. Bittner et al. reported a case involving a 31-year-old substance abuser.[11] The patient accidentally injected a dubious drug into the brachial artery, and, subsequently, acute ischemia developed in the right hand. The patient was successfully treated with intra-arterial urokinase (250.000 IU continuous infusion for 12 hours following 250.000 IU bolus), papaverine (40 mg i.v. 3´4st), heparinization and axillary plexus anesthesia (bupivacaine 0.25%, 10 mL/st).

In the midazolam group in our study, endothelial damage, lamella damage, interstitial oedema and neutrophil infiltration increased from 1 to 15 min, but the increase was statistically significant only at 30 min. These findings show that the damage caused by the injection continues even though the drug has mixed with the circulation.

In a case report by Hering and Angelkort, a patient lost his fingers following intra-arterial injection of a diluted flunitrazepam tablet.[25] This finding supports the finding in our study that damage of the arterial vessel wall progresses with time. In another case report presented by Marsch and Schafer, an intubated patient was accidentally injected with 5 mg midazolam through an arterial pressure line composed of three-way taps during the transfer from an intensive care unit to the operating room.[4] Although the patient, who showed no side effects, was the first case reported, it was stressed that we must be careful regarding the damage and that pressure lines pose risks during transfers.

Considering these data, we chose lidocaine and papaverin, for which the vasodilatation effects are known, as treatment drugs. It has been shown in microvascular tissue-transplant models that topical vasodilator drugs increase blood flow and prevent vasospasm. The two most commonly used drugs are papaverine and lidocaine. Although the vasodilator effects of these two drugs are known, no studies have compared the effect of these drugs in a controlled in vivo model. In a study carried out by Kerschner and Futran, the effect of topical vasodilators on microvascular vessel calibre was examined in a rat model.[15] The effects of papaverine and 1% lidocaine were compared in Sprague-Dawley rats. Both drugs were superior to the saline group, and effects become apparent after 10 min. In this study, papaverine was more efficient in microvascular anastomoses. Our study shows similar results with regard to papaverin. In another study conducted by Evans et al., the vasodilator effect of nicardipine, papaverine and lidocaine on the carotid artery was examined in a rabbit model.[26] Due to the fact that vasospasm was a serious problem in microvascular operations, the response of these three drugs, of which the vasodilator effect was known, was evaluated along with Doppler blood flow. The study showed that nicardipine and papaverine were efficient in increased doses, but due to its partially agonist nature, the effect of lidocaine was variable. We did not collect data on optimal Doppler blood flow, but the effects of papaverine and lidocaine were similar to those observed in this study.

CONCLUSION

In conclusion, endothelial damage, lamella damage, interstitial oedema and neutrophil infiltration increased with time in midazolam-injected rats. Therefore, there is positive correlation between damage and time. By taking into account that proper treatment is related to time, it must be kept in mind that in cases of accidental intra-arterial injections, treatment requires an acute and long time period. Early detection of the injection and the timing of treatment are critical. We have shown that blood vessel wall damage caused by intra-arterial injection of midazolam can be prevented by lidocaine or papaverine in early stages, but long-term treatment may be required. Furthermore, the damage may be histopathologically severe, but the scales of the clinical findings may differ.

REFERENCES

1. Keene JR, Buckley KM, Small S, Geldzahler G. Accidental intra-arterial injection: a case report, new treatment modalities, and a review of the literature. J Oral Maxillofac Surg 2006; 64(6):965-8.
2. Cooper JC, Griffiths AB, Jones RB, Raftery AT. Accidental intra-arterial injection in drug addicts. Eur J Vasc Surg 1992;6(4):430-3.
3. Rees M, Dormandy J. Accidental intra-arterial injection of diazepam. Br Med J 1980;281 (6235):289-90.
4. Marsch SC, Schäfer HG. [An accidental intra-arterial injection of midazolam through a 3-way stopcock in an arterial flushing system]. Anaesthesist 1990;39(6):337-8.
5. Joist A, Tibesku CO, Neuber M, Frerichmann U, Joosten U. [Gangrene of the fingers caused by accidental intra-arterial injection of diazepam]. Dtsch Med Wochenschr 1999;124 (24):755-8.
6. Topazian RG. Accidental intra-arterial injection: a hazard of intravenous medication. J Am Dent Assoc 1970;81(2):410-4.
7. Lieblich SE, Topazian RG. Accidental intra-arterial injection. J Oral Maxillofac Surg 1988; 46(4):297-302.
8. Andreev A, Kavrakov T, Petkov D, Penkov P. Severe acute hand ischemia following an accidental intraarterial drug injection, successfully treated with thrombolysis and intraarterial Iloprost infusion. Case report. Angiology 1995;46(10):963-7.
9. Bhabra MS, Meshikhes AN, Thomson GJ, Craig P, Parrott NR. Intraarterial temazepam: an important cause of limb ischaemia in intravenous drug abusers. Eur J Vasc Surg 1994; 8(2):240-2.
10. Khan ZH, Noorbaksh S. An accidental intra-arterial injection of thiopental on the dorsum of the foot--a case report. Acta Anaesthesiol Taiwan 2004;42(1):55-8.
11. Bittner Ch, Zuber M, Eisner L. [Acute ischemia of the hand in a drug addict after accidental intra-arterial injection]. Swiss Surg 2002;8(6): 281-4.
12. Amrein R, Hetzel W. Pharmacology of Dormicum (midazolam) and Anexate (flumazenil). Acta Anaesthesiol Scand Suppl 1990;92:6-15; discussion 47.
13. Bauer TM, Ritz R, Haberthür C, Ha HR, Hunkeler W, Sleight AJ, et al. Prolonged sedation due to accumulation of conjugated metabolites of midazolam. Lancet 1995;346(8968):145-7.
14. Lei B, Popp S, Capuano-Waters C, Cottrell JE, Kass IS. Effects of delayed administration of low-dose lidocaine on transient focal cerebral ischemia in rats. Anesthesiology 2002;97(6): 1534-40.
15. Kerschner JE, Futran ND. The effect of topical vasodilating agents on microvascular vessel diameter in the rat model. Laryngoscope 1996;106(11):1429-33.
16. Schwartz RS, Huber KC, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. J Am Coll Cardiol 1992;19(2):267-74.
17. Cousins TR, O'Donnell JM. Arterial cannulation: a critical review. AANA J 2004; 72(4): 267-71.
18. Lundy JS. Clinical Anesthesia. A manual of Clinical Anesthesiology. 1st ed. Philedelphia: W.B. Saunders Company; 1942.
19. Dundee JW. Thiopental and other barbiturates. Int Anesthesiol Clin 1956; 34(3): 197-243.
20. Cohen SM. Accidental intra-arterial injection of drugs. Lancet 1948;2(6523):361; passim.
21. Passie T, Metzner C, Seifert J, Zedler M, Schneider U, Emrich HM, et al. [Accidental intraartrial injection of diacethylmorphine (heroin) in drug addicts -- three case reports]. Anasthesiol Intensivmed Notfallmed Schmerzther 2005;40(7):419-22.
22. Treiman GS, Yellin AE, Weaver FA, Barlow WE, Treiman RL, Gaspar MR. An effective treatment protocol for intraarterial drug injection. J Vasc Surg 1990;12(4):456-65; discussion 465-6.
23. Chong M, Davis TP. Accidental intra-arterial injection of propofol. Anaesthesia 1987;42(7): 781.
24. Raininko R. Morphologic endothelial response to meglumine diatrizoate: an electron-microscopic study. Angiology 1981;32(11):733-41.
25. Hering J, Angelkort B. [Acute ischemia of the hand after intra-arterial injection of flunitrazepam. Local combined fibrinolysis therapy in three cases]. Dtsch Med Wochenschr 2006;131(24):1377-80.
26. Evans GR, Gherardini G, Gürlek A, Langstein H, Joly GA, Cromeens DM, et al. Drug-induced vasodilation in an in vitro and in vivo study: the effects of nicardipine, papaverine, and lidocaine on the rabbit carotid artery. Plast Reconstr Surg 1997;100(6): 1475-81.