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Application value of dexmedetomidine and midazolam combined with ultrasound-guided brachial plexus block anesthesia in patients with humeral fractures

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Abstract: This study aims to compare the efficacy and safety of dexmedetomidine and midazolam in patients undergoing humeral fracture surgery. Patients who underwent humeral fracture surgery in our hospital from July 2015 to December 2023 were retrospectively selected. Among the ultrasound-guided brachial plexus nerve block anesthesia protocol, 71 cases used midazolam and were included in Group A, 83 cases used dexmedetomidine and were included in Group B. The fluctuations of heart rate and mean arterial pressure in Group B were smaller than those in Group A. The onset time of sensory and motor block in Group B was shorter than that, while the maintenance time of sensory and motor block was longer. The sedative effect was higher (0.5 hours) after the start of the operation and at the end of the operation in Group B. The pain degree of Group B at 2, 8 and 12 hours after the operation was lower. The cognitive functions in Group B were higher at 30 minutes and 24 hours after the operation. There was no significant difference in the incidence of adverse reactions between the two groups. Dexmedetomidine can be given priority as an anesthetic drug for humeral fracture surgery.

Keywords: Humerus fracture, dexmedetomidine, midazolam, nerve block anesthesia, hemodynamics, sedation analgesia

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INTRODUCTION

Brachial plexus block anesthesia is a widely employed anesthetic technique in humeral fracture surgery. By directly administering local anesthetics around the brachial plexus, it effectively blocks nerve conduction in the innervated region. This method offers several advantages, including simple and reliable operation, high safety and minimal physiological stress (Li *et al*., 2023). The integration of ultrasound-guided technology allows for precise visualization of the brachial plexus anatomy, enhancing puncture accuracy and enabling real-time monitoring of local anesthetic spread, this ultimately reinforces the security and effectiveness of the entire process (Zhao *et al*., 2023). However, patients often experience anxiety and fear due to pain from fractures and concerns about surgical risks, which can lead to emotional instability and incomplete nerve block, potentially compromising surgical outcomes (Kaye *et al*., 2020). Consequently, sedative adjuvant drugs are frequently used in clinical practice to enhance anesthetic effectiveness. Despite this, there remains no standardized protocol for medication selection. Midazolam and dexmedetomidine are frequently employed as sedative adjuncts during surgical operations. Midazolam, a classic benzodiazepine, exhibits rapid and reliable sedative-hypnotic effects, effectively reducing preoperative anxiety and enhancing patient cooperation, thereby optimizing the overall surgical experience (Xu *et al*., 2021). Dexmedetomidine, a novel α2-adrenergic receptor agonist, provides excellent sedative and analgesic effects along with anti-sympathetic activity, which helps to reduce surgical stress responses, maintain hemodynamic stability and alleviate postoperative pain (Ghasemi *et al*., 2023). Given their distinct characteristics and potential advantages, this study utilized real-world data from electronic medical records to evaluate and contrast the effectiveness and safety profile of midazolam and dexmedetomidine when combined with ultrasound-guided brachial plexus block anesthesia in patients with humeral fractures, aiming to provide a reference for clinical decision-making regarding sedative adjuvant drugs.

MATERIALS AND METHODS

Research subjects

A retrospective cohort study design was adopted. Patients who underwent humeral fracture surgery in our hospital from July 2015 to December 2023 were selected as the study population. According to different anesthesia regimens, patients who received midazolam combined with ultrasound-guided brachial plexus block anesthesia were classified as Group A (n=71) and those who received dexmedetomidine combined with ultrasound-guided brachial plexus block anesthesia were classified as Group B (n=83). The Institutional Medical Ethics Committee endorsed our study.

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Inclusion criteria included: (1) patients diagnosed with humeral fracture via imaging and who successfully completed surgery (Rudran *et al.*, 2022); (2) aged 18 years or older; (3) comprehensive medical documentation. Exclusion criteria included: (1) a history of previous humeral fractures or surgeries; (2) concurrent metabolic bone diseases, hematological disorders, or liver and kidney dysfunction; (3) use of other primary anesthesia methods.

Sample size calculation: The sample size formula for comparing the means of two independent samples was adopted and the anesthetic block effect was used as the main outcome measure for calculation. Based on literature review and practical experience, it is estimated that the time difference (δ) for the onset of block between the two groups is 2 minutes, the standard deviation (σ) is 3, the detection efficacy α=0.05 and 1-β=0.95. Considering a loss to follow-up rate of 10%, at least 66 cases are needed in each group. The calculation formula is as follows.

$$N = 2 \times \left[\frac{\left(Z_{1-\alpha/2} + Z_{1-\beta} \right) \times \sigma}{\delta} \right]^2$$

Anesthesia methods

Upon entering the surgical suite, set up peripheral venous access and routine anesthesia monitoring was initiated. For Group A, intravenous anesthesia with midazolam (Jiangsu Enhua Pharmaceutical, 10mL: 50mg) was adopted. The initial dose was a slow intravenous injection of 0.03-0.04mg/kg, followed by an additional 0.01-0.02mg/kg approximately every 5 minutes for maintenance. The total dose did not exceed 0.1mg/kg (Hong *et al*., 2021). For Group B, dexmedetomidine (Jiangsu Enhua Pharmaceutical, 2mL:0.2mg) was infused via micro-pump. The initial infusion rate was set at 0.5μg/(kg·h) for 10 minutes, followed by a maintenance dose of 0.3μg/(kg·h). Dexmedetomidine administration was discontinued 10 minutes before the end of surgery (Baek *et al*., 2023). Fifteen minutes after drug administration, the patient received an anesthesia via ultrasound-guided brachial plexus block. Patients were positioned in a lying-down posture and the anesthesia site was routinely disinfected. Using an ultrasound diagnostic system with a high-frequency linear probe positioned at the interscalene groove, the end of the needle was passed through the intermediate scalene muscle to reach the deep part of the lower trunk. When the needle tip was observed at the upper, middle and lower positions of the brachial plexus within the interscalene indentation, it was advanced using ultrasound guidance until it reached the perineural area. Needle advancement was halted upon confirmation of proper positioning on the ultrasound monitor. After ensuring no cerebrospinal fluid, air, or blood on aspiration, 25-30 mL of a mixture of 0.75% ropivacaine (Shijiazhuang Sihui Pharmaceutical, 10mL:100mg) and 1% lidocaine (Shanghai Zhaohui Pharmaceutical, 10mL:0.2g) was injected (Hong *et al*., 2019).

Observation indicators

Hemodynamics

The time points were defined as follows: T0 (entered the operating room), T1 (10 minutes after surgery began), T2 (at the moment of skin incision), T3 (30 minutes after surgery started) and T4 (the conclusion of surgery). Heart rate (HR) and mean arterial pressure (MAP) at these five time intervals were compared among the both groups.

Block effect

The sensory and motor obstruction effectswere assessed and a comparison was made among the both groups. Onset of sensory block was characterized by the time from drug administration to the cessation of pain sensation in the blocked area upon needle puncture, while sensory block duration was determined as the duration from drug administration until the patient reported pain at the surgical site (Sane *et al*., 2021). Motor block onset was determined as the period from when the drug was administered to the loss of motor function, including thumb abduction, adduction, opposition and elbow flexion, while the duration of motor block was determined as the time from drug administration until the patient's motor function was restored to normal (Shukla *et al*., 2023).

Sedative effect

The effects at various time points (T1 to T4) in the two groups were compared using the Ramsay sedation scale (Sachdeva *et al*., 2023). The scale ranges from 1 to 6, with the following definitions: 1=restlessness, 2=alert and cooperative, 3=somnolent but reacts to instructions, 4=light sleep yet easily roused, 5=asleep with a slow response to vocal stimuli and 6=Profound sleep with no reaction to auditory stimuli. Scores of 1-2 indicate unsatisfactory sedation, 3-4 indicate satisfactory sedation and 5-6 indicate excessive sedation.

Analgesic effect: The Numerical rating scale (NRS) (Tore *et al*., 2023) was utilized for assess pain levels in both groups at four time points: before anesthesia, 2 hours postoperatively, 8 hours postoperatively and 12 hours postoperatively. The NRS ranges from 0 to 10, where 0 indicates no pain and 10 indicates severe pain. Higher scores indicate more intense pain.

Cognitive function: The Mini-mental state examination (MMSE) (Jiayuan *et al*., 2022) was used to assess and compare cognitive function levels between the two groups at four time points: before anesthesia, 30 minutes postoperatively, 24 hours postoperatively and 48 hours postoperatively. The MMSE assesses six areas: orientation, memory, attention, calculation, recall and language function, with a maximum possible score of 30. Higher scores are associated with superior cognitive performance.

Adverse reactions

The incidence of adverse reactions, including

postoperative coughing, bradycardia, restlessness, nausea and vomiting and elevated blood pressure, was compared between the two groups following anesthesia.

STATISTICAL ANALYSIS

Data analysis was performed with the SPSS 26.0. For quantitative data that conformed to a Gaussian distribution, results were represented as mean ± standard deviation ($\bar{x} \pm s$) and comparisons between different groups were conducted using independent-sample t-tests. For quantitative data that did not conform to a Gaussian distribution, results were presented as median with quartile range [M(Q_{25}, Q_{75})] and the Wilcoxon rank sum test was applied. Repeated measures analysis of variance (ANOVA) was employed to compare measurement data at different time points, using Bonferroni correction for back testing. Frequency data were displayed as the count of cases and proportion [n(%)] and χ^2 tests were utilized for contrasts. Regarding ordinal data, the Wilcoxon rank sum test was applied to analyze. P<0.05 was deemed to indicate statistical significance.

RESULTS

Analysis of baseline data characteristics

The two groups did not show any statistically significant differences with respect to age, gender, body mass index (BMI), hypertension, fracture location, Association for the Study of Internal Fixation of Fractures (AO) type and American Society of Anesthesiologists (ASA) classification (P>0.05), as presented in table 1.

Analysis of hemodynamic parameters

At T1, T2, T3 and T4, the HR and MAP values in both groups were significantly reduced compared to those at T0 (P<0.05). In Group B, the HR was markedly elevated at T1, T3 and T4 in comparison to Group A; Conversely, at T2, the HR was notably lower than that observed in Group A (P<0.05). The group's primary impact demonstrated statistical significance (F_{group}=13.571, P<0.05), as did the primary impact of the measurement time points (F_{time}=67.010, P<0.05). Moreover, the interaction between the group and the measurement time points also demonstrated significance ($F_{group·time}$=14.961, P<0.05), see table 2. In Group B, the MAP values at T2, T3 and T4 were markedly elevated compared to those in Group A (P<0.05).

The group's primary impact demonstrated statistical significance (F_{group}=26.934, P<0.05), as did the primary impact of the measurement time points (F_{time}=57.918, P<0.05). Moreover, the interaction between the group and the measurement time points also demonstrated significance ($F_{group·time}$=5.581, P<0.05), see table 3. Based on HR and MAP, the variation range of Group B at each time point was relatively smaller than that of Group A and the variation of Group B was more stable, as shown in fig. 1A-B.

Analysis of block effects

In comparison to Group A, Group B exhibited a reduced onset time for both sensory and motor block, yet a prolonged duration for both sensory and motor block (P<0.05), see table 4.

Analysis of sedation effects

The Ramsay sedation scores in both groups ranged from 1 to 4. There were no significant differences in Ramsay sedation scores between groups A and B at T1 and T2 (P>0.05). However, at T3 and T4, Group B exhibited significantly higher Ramsay sedation scores compared to Group A (P<0.05), as illustrated in fig. 2A-D.

Analysis of analgesic effects

No significant difference was observed in NRS scores between the two groups prior to anesthesia (P>0.05). At 2, 8 and 12 hours after surgery, patients in Group B reported markedly reduced NRS scores than those in Group A (P<0.05), as illustrated in fig. 3A-D.

Analysis of cognitive functions

The MMSE scores showed no significant difference comparing the two groups prior to anesthesia and 48 hours after the surgery (P>0.05). However, Group B exhibited significantly higher MMSE scores than Group A at 30 minutes and 24 hours postoperatively. The group's primary impact demonstrated statistical significance (F_{group}=14.242, P<0.05), as did the primary impact of the measurement time points (F_{time}=23.520, P<0.05). Moreover, the interaction between the group and the measurement time points also demonstrated significance ($F_{group·time}$=4.033, P<0.05), see table 5.

Analysis of adverse reactions

In Group A, the occurrence rate of adverse reactions was 15.49% and in Group B it was 8.43%. The rate of adverse effects occurrence did not show a statistically significant difference in the two groups (P>0.05), see table 6.

DISCUSSION

In recent years, ultrasound-guided technology has advanced rapidly, significantly enhancing the precision of brachial plexus block and improving anesthetic outcomes. However, as a form of regional anesthesia, brachial plexus block allows patients to perceive surgical procedures, potentially leading to anxiety, fear and heightened stress responses. This can result in significant hemodynamic fluctuations, compromising surgical safety.

Additionally, the administration of supplemental anesthetics during surgery may increase the risk of over-sedation. Therefore, optimizing the anesthetic protocol for humeral fracture surgery is critically important.

Table 1: Analysis of baseline data characteristics

Data	Group A (n=71)	Group B (n=83)	$t/\chi^2/Z$	P
Age (years, $\bar{x} \pm s$)	61.58±13.35	62.96±9.24	0.737	0.463
Gender [n (%)]			0.452	0.501
Male	44 (61.97)	47 (56.63)		
Female	27 (38.03)	36 (43.37)		
BMI (kg/m^2, $\bar{x} \pm s$)	22.75±2.41	23.38±2.24	1.693	0.093
Hypertension [n (%)]	30 (42.25)	24 (28.92)	2.990	0.084
Fracture location [n (%)]			0.041	0.980
Proximal humeral fractures	17 (23.94)	20 (24.10)		
Fracture of the humeral shaft	29 (40.85)	35 (42.17)		
Distal humeral fracture	25 (35.21)	28 (33.73)		
AO type [n (%)]			0.256	0.798
Fracture location [n (%)]			0.041	0.980
Proximal humeral fractures	17 (23.94)	20 (24.10)		
Type A	28 (39.44)	36 (43.37)		
Type B	20 (28.17)	31 (37.35)		
Type C	23 (32.39)	26 (31.33)		
ASA classification [n (%)]			0.658	0.511
I	22 (30.99)	24 (28.92)		
II	40 (56.34)	44 (53.01)		
III	9 (12.68)	15 (18.07)		

Note: BMI: body mass index; AO: association for the study of internal fixation of fractures; ASA: american society of anesthesiologists

Table 2: Comparison of HR at different times in two groups (beats/min, $\bar{x} \pm s$)

	T0	T1	T2	T3	T4
Group A	75.41±4.71	71.25±4.98	73.29±5.42	66.31±4.04	67.00±4.60
Group B	75.46±3.69	72.90±4.40[a]	70.48±4.79[a]	69.61±3.16[a]	70.84±4.69[a]
F_{group}/P			13.571/<0.001		
F_{time}/P			67.010/<0.001		
$F_{group\cdot time}/P$			14.961/<0.001		

Note: T0: entered the operating room; T1: 10 minutes after surgery began); T2: at the moment of skin incision; T3: 30 minutes after surgery started; T4: the conclusion of surgery; Compared with Group A, [a] indicates P<0.05

Table 3: Comparison of MAP at different Times in two groups (mmHg, $\bar{x} \pm s$)

	T0	T1	T2	T3	T4
Group A	102.55±6.26	97.49±5.14	92.24±7.59	93.13±4.33	90.37±7.68
Group B	102.72±7.16	97.23±6.78	98.06±6.46[a]	95.28±6.21[a]	93.84±5.95[a]
F_{group}/P			26.934/<0.001		
F_{time}/P			57.918/<0.001		
$F_{group\cdot time}/P$			5.581/<0.001		

Note: T0: entered the operating room; T1: 10 minutes after surgery began); T2: at the moment of skin incision; T3: 30 minutes after surgery started; T4: the conclusion of surgery; Compared with Group A, [a] indicates P<0.05

Table 4: Analysis of onset and duration times for sensory and motor block (min, $\bar{x} \pm s$)

	Sensory block		Motor block	
	Onset time	Duration time	Onset time	Duration time
Group A	15.10±3.28	478.85±43.51	16.87±4.77	549.94±52.77
Group B	12.05±3.66	624.70±29.88	12.27±3.91	677.55±49.65
t	5.406	23.843	6.485	15.445
P	<0.001	<0.001	<0.001	<0.001

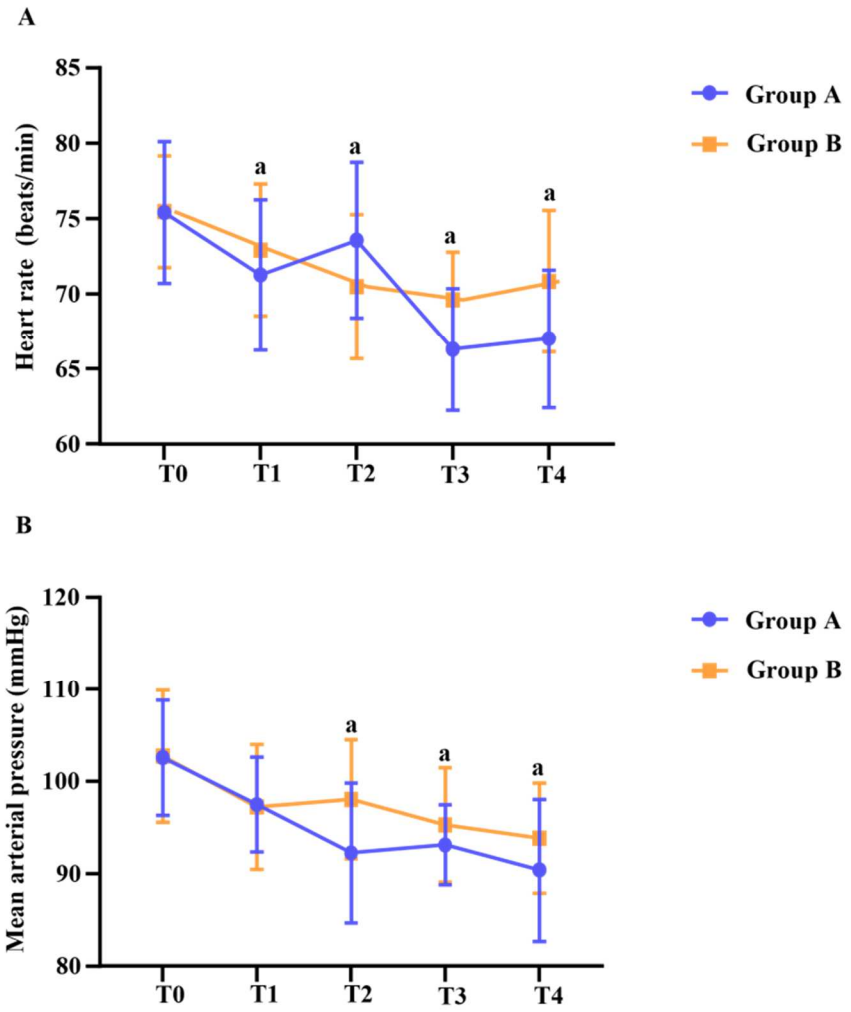

Fig. 1: Comparison of heart rate (HR) and mean arterial pressure (MAP) at different time points between the two groups. Compared with Group A, [a] indicates $P<0.05$. A: Comparison of HR between the two groups; B: Comparison of MAP between the two groups

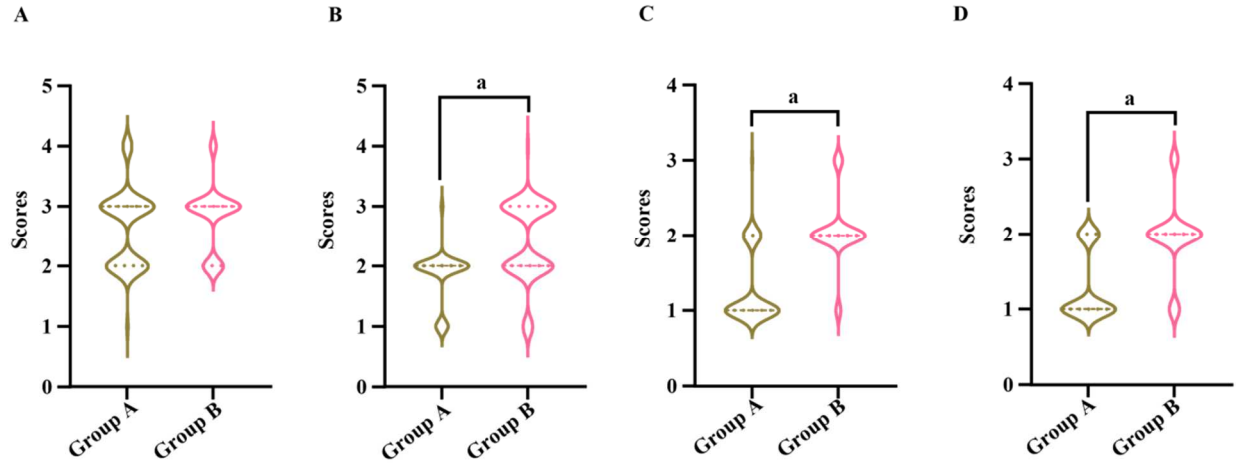

Fig. 2: Comparison of Ramsay sedation scores at different time points between the two groups. Compared with Group A, [a]indicates $P<0.05$. A: Ramsay sedation scores at T1; B: Ramsay sedation scores at T2; C: Ramsay sedation scores at T3; D: Ramsay sedation scores at T4

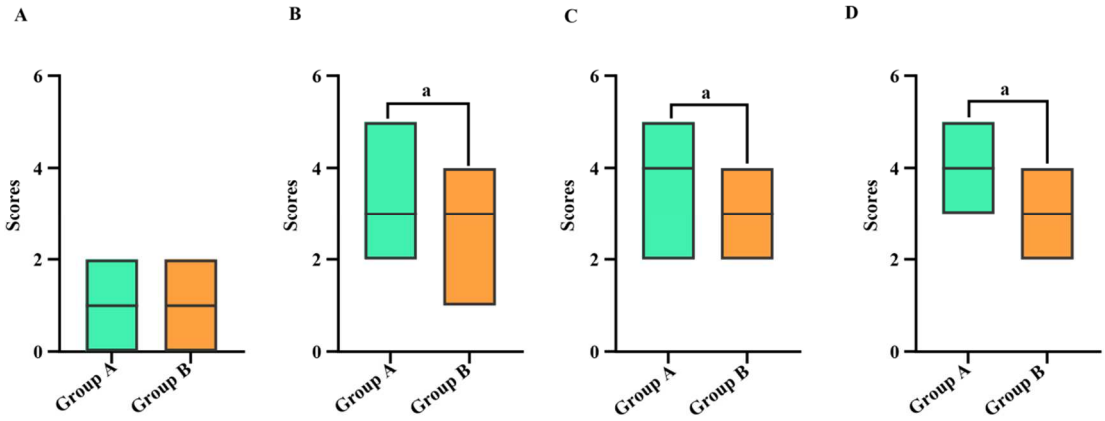

Fig. 3: Comparison of Numerical rating scale (NRS) scores at different time points between the two groups. Compared with Group A, [a]indicates $P<0.05$. A: NRS scores before anesthesia; B: NRS scores 2 hours postoperatively; C: NRS scores 8 hours postoperatively; D: NRS scores 12 hours postoperatively

Table 5: Analysis of MMSE scores at different times (Points, $\bar{x} \pm s$)

	Before anesthesia	30 minutes postoperatively	24 hours postoperatively	48 hours postoperatively
Group A	23.06±3.88	19.79±5.03	22.63±3.54	24.00±3.36
Group B	22.66±3.50	22.12±4.11[a]	24.34±2.34[a]	24.70±3.04
F_{group}/P		14.242/<0.001		
F_{time}/P		23.520/<0.001		
$F_{group \cdot time}/P$		4.033/<0.01		

Note: Compared with Group A, [a] indicates $P<0.05$

Table 6: Analysis of adverse reactions [n (%)]

	Coughing	Bradycardia	Restlessness	Nausea / Vomiting	Elevated blood pressure	Total
Group A	3 (4.23)	1 (1.41)	2 (2.82)	0 (0.00)	5 (7.04)	11 (15.49)
Group B	1 (1.20)	2 (2.41)	0 (0.00)	1 (1.20)	3 (3.61)	7 (8.43)
χ^2						1.847
P						0.174

In the past, ultrasound-guided brachial plexus nerve block anesthesia was frequently used in conjunction with intravenous midazolam. Midazolam specifically binds to benzodiazepine receptors, mediating the opening of chloride ion channels in related neurons. This accelerates chloride ion influx, leading to neuronal hyperpolarization and central nervous system inhibition, thereby exerting hypnotic and sedative effects (Zuo *et al*., 2024). While midazolam has a rapid onset and quick distribution throughout the body, its short duration necessitates multiple dose increments during surgery. This can lead to adverse events such as respiratory depression and excessive sedation, increasing perioperative risks (Morimoto *et al.*, 2022). Additionally, midazolam lacks significant analgesic properties, which may not adequately mitigate the stress response and could potentially compromise the overall anesthetic effect (Wang *et al*., 2023). With the advancement of pharmacological research, dexmedetomidine, an efficient novel sedative adjuvant, has garnered increasing attention for its combined use with ultrasound-guided brachial plexus block anesthesia. Compared to midazolam, dexmedetomidine exhibits a more rapid onset, superior analgesic properties, greater pharmacological stability, a relatively longer elimination half-life and does not necessitate frequent dosage adjustments. Additionally, it exerts a weaker inhibitory effect on the respiratory system (Baek S *et al*., 2023, Hong *et al.*, 2019).

This study compared the effects of combining two different sedatives with ultrasound-guided brachial plexus block anesthesia in patients who were having surgery for humeral fractures. The results are as follows: (1) In Group B, the trends of HR and MAP changes at T1, T2, T3 and T4 were more stable compared to those in Group A, indicating that dexmedetomidine provides superior hemodynamic stability. This can be attributed to the high selectivity of dexmedetomidine for α2-adrenergic receptors. By selectively activating these receptors in the central locus coeruleus, dexmedetomidine reduces sympathetic nerve activity and inhibits the release of norepinephrine, thereby inducing a quasi-sleep state. This mechanism helps

maintain cardiovascular stability and minimizes the impact on the respiratory system, resulting in more stable hemodynamics (Eizaga *et al*., 2022). (2) In Group B, the onset time for both sensory and motor block was markedly reduced, while the maintenance time was notably longer, indicating that dexmedetomidine can enhance the effectiveness of anesthesia block. Kumar G *et al*. (Kumar *et al*., 2018) demonstrated that in upper limb surgery, compared with midazolam, intravenous dexmedetomidine for brachial plexus block accelerates the onset and prolongs the duration of both sensory and motor block. This effect is attributed to dexmedetomidine's ability to block hyperpolarization-activated cyclic nucleotide-gated cation channels, thereby exerting non-receptor-dependent analgesic effects. By enhancing activity-dependent hyperpolarization and reducing nociceptive synaptic transmission in the spinal cord dorsal root ganglia, it strengthens the nerve block by inhibiting unmyelinated C fibers resistant to local anesthetics, thus prolonging the block duration (Chen *et al*., 2023, Sane *et al*., 2021). Additionally, after absorption into the bloodstream, dexmedetomidine binds to α2-adrenergic receptors, activating α2 receptors in vascular smooth muscle, inhibiting the release of substance P from the spinal cord dorsal root ganglia, promoting vasoconstriction and prolonging the metabolism and absorption time of local anesthetics. This process inhibits the transfer of pain signals toward the cns depression, further extending the duration of nerve block (Xiong *et al*., 2021). (3) Compared with Group A, Group B exhibited higher Ramsay sedation scores at T3 and T4 during surgery and lower NRS pain scores at 2, 8 and 12 hours postoperatively, indicating superior sedative and analgesic effects of dexmedetomidine. Hong B *et al*. (Hong *et al*., 2019) demonstrated that, In contrast to midazolam, dexmedetomidine substantially extends the duration of analgesia during brachial plexus block for upper limb orthopedic surgery. This effect can be attributed to dexmedetomidine's ability to increase affinity for α2-adrenergic receptors and enhance intrinsic activity, thereby exerting significant sedative and anxiolytic effects. Dexmedetomidine acts on presynaptic and postsynaptic α2-adrenergic receptors in the spinal cord's dorsal horn neurons, inhibiting pain signal transmission. Additionally, it directly affects the locus coeruleus in the brainstem, which regulates wakefulness and sleep, maintaining patients in a natural non-rapid eye movement state. This mechanism contributes to achieving optimal sedative and analgesic outcomes (Donatiello *et al.*, 2022, Ye *et al*., 2024).

In terms of safety, Group B exhibited higher MMSE scores at 30 minutes and 24 hours postoperatively compared to Group A, indicating that dexmedetomidine is beneficial for early cognitive recovery after surgery. This effect may be attributed to dexmedetomidine's neuroprotective properties, which include reducing glutamate concentrations, alleviating brain injury, protecting hippocampal neurons and enhancing cerebral oxygenation. By mitigating cerebral ischemia and hypoxia, dexmedetomidine helps reduce the incidence of postoperative delirium, thereby safeguarding cognitive function (Li *et al*., 2020, Huang *et al*., 2024). Additionally, the overall rate of adverse effects did not significantly differ between the two groups, indicating that dexmedetomidine combined with ultrasound-guided brachial plexus block anesthesia does not elevate the likelihood of adverse effects and demonstrates a favorable safety profile. This may be attributed to the relatively low dosage of dexmedetomidine, its administration via micro-pump infusion and its rapid elimination from the body after discontinuation without significant drug accumulation. These factors contribute to enhanced safety and reduce the likelihood of adverse effects (Cai *et al*., 2022).

However, this study had several limitations. The relatively small sample size and reliance on retrospective medical records may have introduced incomplete information or recording biases, potentially affecting the accuracy and reliability of the findings. While multiple aspects were compared, important clinical indicators or complications may not have been fully captured, limiting the comprehensiveness and depth of the results. Therefore, future research should adopt a prospective, multi-center, large-sample design with strict control of relevant variables to more accurately assess the application value of the two anesthesia protocols in patients with humeral fractures.

CONCLUSION

In conclusion, compared with midazolam, dexmedetomidine combined with ultrasound-guided brachial plexus block anesthesia in patients with humeral fractures offers superior hemodynamic stability, faster onset of nerve block, prolonged block duration, enhanced sedative and analgesic effects and promotes early cognitive recovery after surgery without raising the rate of adverse effects. These advantages make it a promising option for clinical application and warrant further promotion.

Consent to participate

We secured a signed informed consent form from every participant.

Ethical approval

This study was approved by the Ethics Committee of the Shaoxing Shunji Hospital (YK--20250187).

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contribution

Dongjing Huo, Caizhi Zhang: Developed and planned the study, performed experiments and interpreted results.

Edited and refined the manuscript with a focus on critical intellectual contributions.
Lijie Li: Participated in collecting, assessing and interpreting the date. Made significant contributions to date interpretation and manuscript preparation.
Rong Zeng: Provided substantial intellectual input during the drafting and revision of the manuscript.

Conflicts of interest
The authors declare that they have no financial conflicts of interest.

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