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Research Article

SCALP Block Model in Sprague-Dawley Rats: In Vivo Experimental Study and Model Identification

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Abstract **Introduction:** The SCALP block is an important regional anesthesia method. In this study, we analyzed the SCALP block model for the first time in a rat model. **Materials and Methods:** In this study, 16 rats were divided into four different groups. Six nerve blocks were planned in both halves of the skull for the SCALP block, and three entry points were determined for each. Bupivacaine (Marcaine, AstraZeneca) 0.5 mg/kg was injected in the "low-dose" group, 1 mg/kg in the "medium-dose" group, 2 mg/kg in the "high-dose" group, and 4 mg/kg in the "very high-dose" group. A midline fronto-occipital incision was made, and a craniectomy was performed. At 0, 30, 60, 90, and 120 minutes after the surgical procedure, the rats were evaluated using the Rat Grimace Scale, and their pain scores were evaluated. At the end of 2 hours, the rats were sacrificed, blood samples were taken, and methemoglobin levels were studied. Finally, a comparative analysis was performed between the four groups. **Results:** In 30, 60, 90, and 120 minutes after surgery, the differences between the grimace scores between low-dose and medium-dose and high-dose and low-dose groups were statistically significant ($p<0.001$). There was no significant difference between the high-dose and very high-dose groups regarding the grimace scale ($p>0.05$). Methemoglobin levels were statistically significantly higher after SCALP block with a very high dose of bupivacaine than blockade with a high dose of bupivacaine ($p<0.001$). Therefore, very high-dose bupivacaine injection was not superior to high-dose bupivacaine injection in pain control. In addition, very high doses of bupivacaine injection can be associated with increased morbidity and mortality due to increased methemoglobin levels. **Conclusion:** In this SCALP block technique described in rats, a 2 mg/kg bupivacaine injection can be considered an appropriate dosage for both safety and efficacy.

Keywords SCALP block; Sprague-Dawley rats; Regional anesthesia; Model

Introduction

Regional anesthesia is a safe anesthetic method that can bypass the systemic side effects and complications of general anesthetic agents (1). Following the emergence of regional anesthesia techniques, significant improvements have been experienced in durations, postoperative outcomes, and remission processes of several surgeries. Many studies are showing that SCALP block, which is a type of regional anesthesia, is an alternative option in patients with heart failure, lung diseases, and metabolic syndrome where the administration of systemic anesthetic agents is at high risk, as well as reducing the need for opioids in the postoperative period in patients (1,2). Patel et al. published a case series showing that SCALP block significantly reduced postoperative opioid use (1). Another case-control study by Festa et al. demonstrated the efficacy of SCALP block in postoperative pain control in patients with craniosynostosis (2).

Procedures requiring craniotomy constitute an essential part of neurosurgery practice (3-5). One of the significant steps of the rehabilitation process after craniotomy is pain control. Postoperative pain may cause hypertension and tissue edema through sympathetic discharge. This condition adversely affects wound healing through tissue edema and increases the risk of intracranial hemorrhage in the surgical site in the postoperative period (3-5). In this respect, proper pain control is essential in ensuring postoperative patient comfort and improving surgical outcomes.

Animal research has become one of the critical components of scientific progress recently. Experimental animal models in vivo studies help investigate problems that may be encountered in the clinic. In many models, such as the experimental traumatic subarachnoid hemorrhage model and experimental spinal cord ischemia-reperfusion model, rats, rabbits, and mice are used as subjects (6, 7). However, the literature has previously described no method for SCALP block in rats. This study aimed to prepare a protocol to create a model of SCALP block in rats.

Materials and Methods

This experiment was carried out in Ankara NeSa Animal Research Laboratory. The Ankara NeSa Animal Research Laboratory Ethics Committee reviewed the research protocol, and the ethics committee approval was obtained (10.01.2023/003). In addition, all animals received care as per "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Institute of Health.

Rats were divided into four equal groups: Low-dose, medium-dose, high-dose, and very high-dose, with four rats in each group.

Animal Care

The animals were kept at room temperature between 18-21 degrees Celcius. The rats were injected intramuscularly with xylazine 5 mg/kg (Rompun, Bayer) as a muscle relaxant and allowed to breathe spontaneously.

The SCALP Block Technique

The supraorbital, supratrochlear, posterior auricular, temporal, zygomatic, major, and minor occipital nerves are targeted in this technique. For targeting, three separate injection points were determined for both halves of the skull. For the first injection point, the end of the orbital rim was determined at 3 mm posterior to the mid-pupillary line. Next, the supraorbital and supratrochlear nerve blockade was targeted by entering a 30G insulin injector at a 45-degree angle (Figure 1). The second injection point was determined as the junction of the posterior zygomatic arch 2 mm anterior and inferior to the ear tragus. Next, the posterior auricular, temporal, and zygomatic nerves were blocked by an oblique entry with a 30G insulin injector (Figure 1). The third injection point was determined as the junction of the occipital protuberance, 4 mm lateral to the mid-sagittal line posteriorly. Finally, the minor and major

occipital nerve blocks were targeted by entering with a 30G insulin injector at a 45-degree angle (Figure 1). No sedation was performed during the procedure.

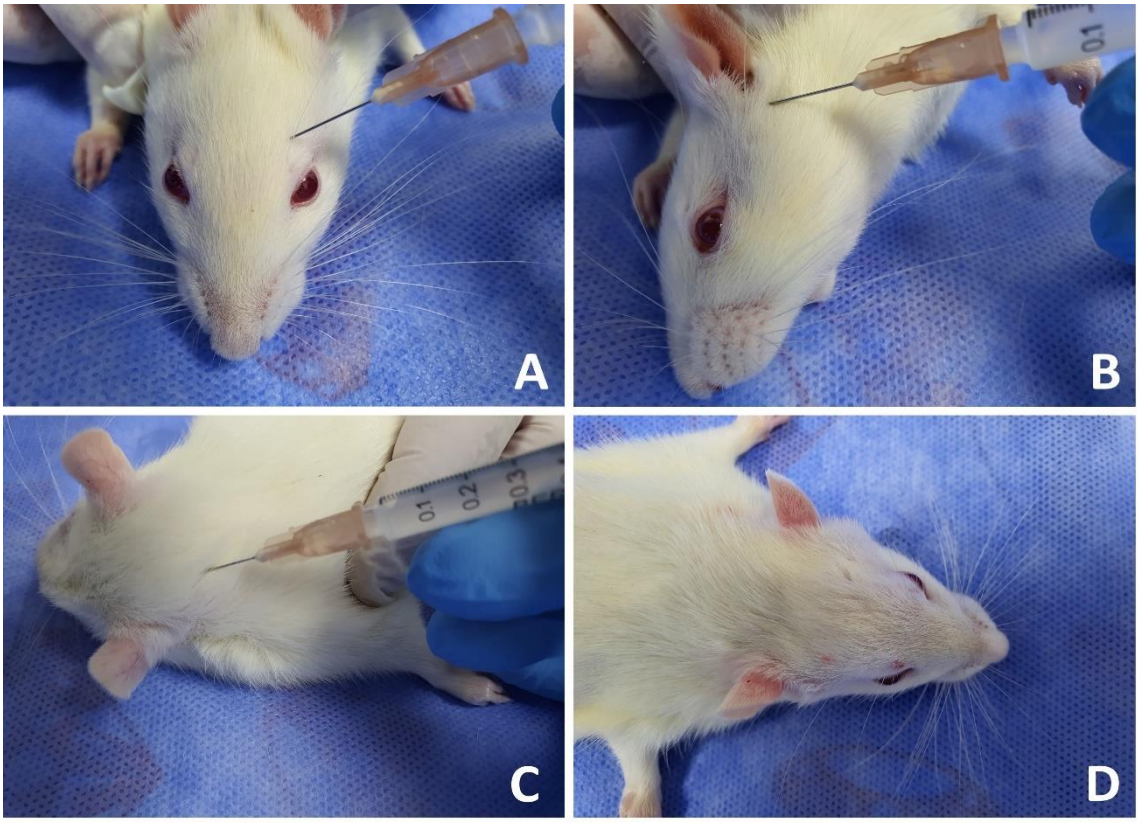

Figure 1. Injection points for SCALP block model. A: The first entry point. The end of the orbital rim was determined at 3 mm posterior to the mid-pupillary line and targeted by entering a 30G insulin injector at a 45-degree angle. B: The second entry point. The junction of the posterior zygomatic arch 2 mm anterior and inferior to the ear tragus was entered obliquely with a 30G insulin injector. C: The third entry point. The junction of the occipital protuberance, 4 mm lateral to the midsagittal line posteriorly, was targeted by entering with a 30G insulin injector at a 45-degree angle. D: Prone position after SCALP block.

Surgical Technique

A 6-point SCALP block protocol was performed on each animal in four groups. For the block, 0.5 mg/kg bupivacaine (Marcaine, AstraZeneca) was administered in the low-dose group, 1 mg/kg in the medium-dose group, 2 mg/kg in the high-dose group, and 4 mg/kg in the very high-dose group.

After bilateral SCALP block with a 6-point injection protocol and intramuscular injection of xylazine, animals were placed in the prone position (Figure 1). After the scalp was shaved, the skin was dissected by a linear incision in the fronto-occipital plane (Figure 2). After the periosteum was scraped, fronto-occipital craniectomy was performed (Figure 2). Subsequently, the subcutaneous layer and skin were sutured and closed (Figure 2). After closure, the rats were placed in cages and recorded with a camera. Pain scores of the rats were performed using the Rat Grimace Scale (RGS) at 0, 30, 60, 90, and 120 minutes postoperatively (6). At the end of the second hour, the rats were sacrificed by intracardiac puncture and aspiration, and blood samples were collected.

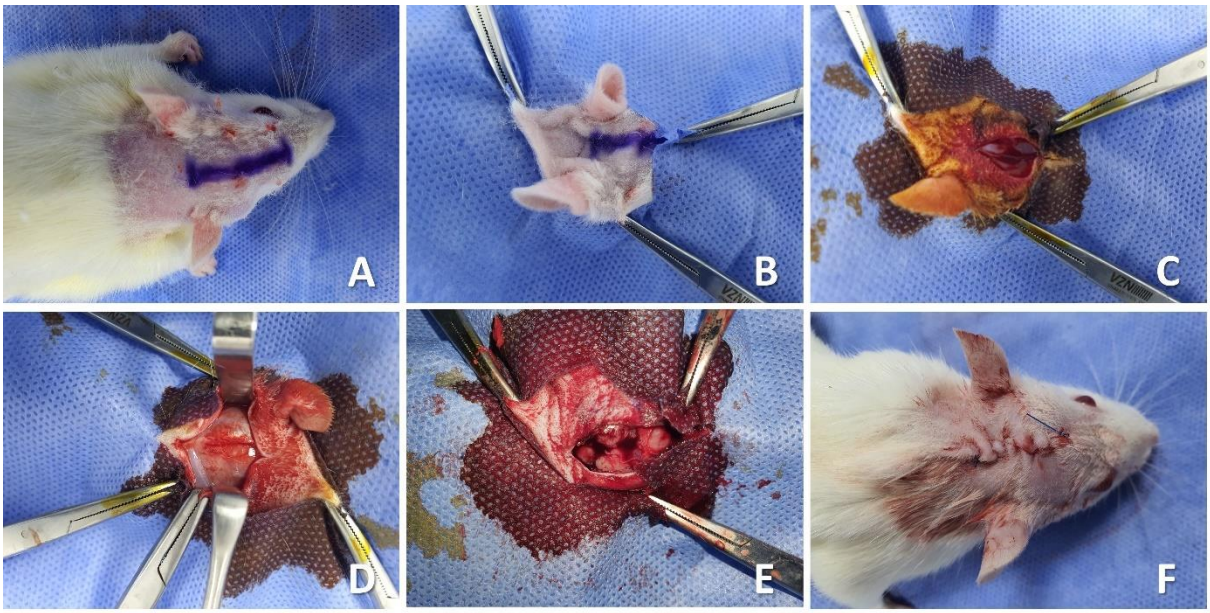

Figure 2. Surgical procedures. A: Shaving the scalp B: Preparing surgical area by draping C: Linear incision in the fronto-occipital plane D: Removal of the periosteum E: Craniectomy procedure F: Primary closure of the skin

Biochemical Evaluation

Blood gas and methemoglobin levels were studied to evaluate bupivacaine's toxicity.

Statistical Analysis

Data collected in groups were analyzed using IBM SPSS Statistics Version 25. The one-way ANOVA test was used to determine parametric differences between groups. In addition,

descriptive analysis for one numeric variable was used to create plots. Data were presented as means and standard deviations. The confidence interval (CI) was computed as 95%, and the difference was considered significant when the p-value was lower than 0,05.

Results

Sixteen Sprague-Dawley weighing 250-350 grams male rats were used. Each group included four rats. In the very high-dose group, 2 of the rats died 30 minutes after completion of the surgical procedure, and their blood samples were taken immediately after death.

The comparative analysis found a statistically significant difference between the low-dose group and the other three groups in terms of the 0-minute, 30-minute, 60-minute, 90-minute, and 120-minute RGS scores ($p<0.001$). A statistically significant difference was found in the comparison of the medium-dose group with the high-dose and very high-dose groups ($p<0.001$). When blood methemoglobin levels were compared, a statistically significant difference was observed between low-dose, medium-dose, high-dose, and very high-dose groups ($p<0.001$) (Figure 3). Since deaths were detected in the very high-dose group, 4 mg/kg was considered a lethal dose in the SCALP block.

In 30, 60, 90, and 120 minutes after surgery, RGS scores were significantly between the low-dose and medium-dose, medium-dose and high-dose, and low-dose and high-dose groups ($p<0.001$) (Figure 3). However, there was no significant difference between the high-dose gand very high-dose groups in terms of RGS scores ($p>0.05$) (Figure 3). From this point of view, it can be suggested that the performance of SCALP block with very high-dose bupivacaine does not contribute to the improvement in the pain scale compared with blockade using high-dose bupivacaine. In addition, methemoglobin levels were significantly higher after SCALP block with very high-dose bupivacaine compared to blockade with high-dose bupivacaine ($p=0.002$) (Figure 4).

Thus, it can be suggested that very high doses of bupivacaine used for SCALP block may increase the risk of morbidity and mortality due to increased methemoglobin levels.

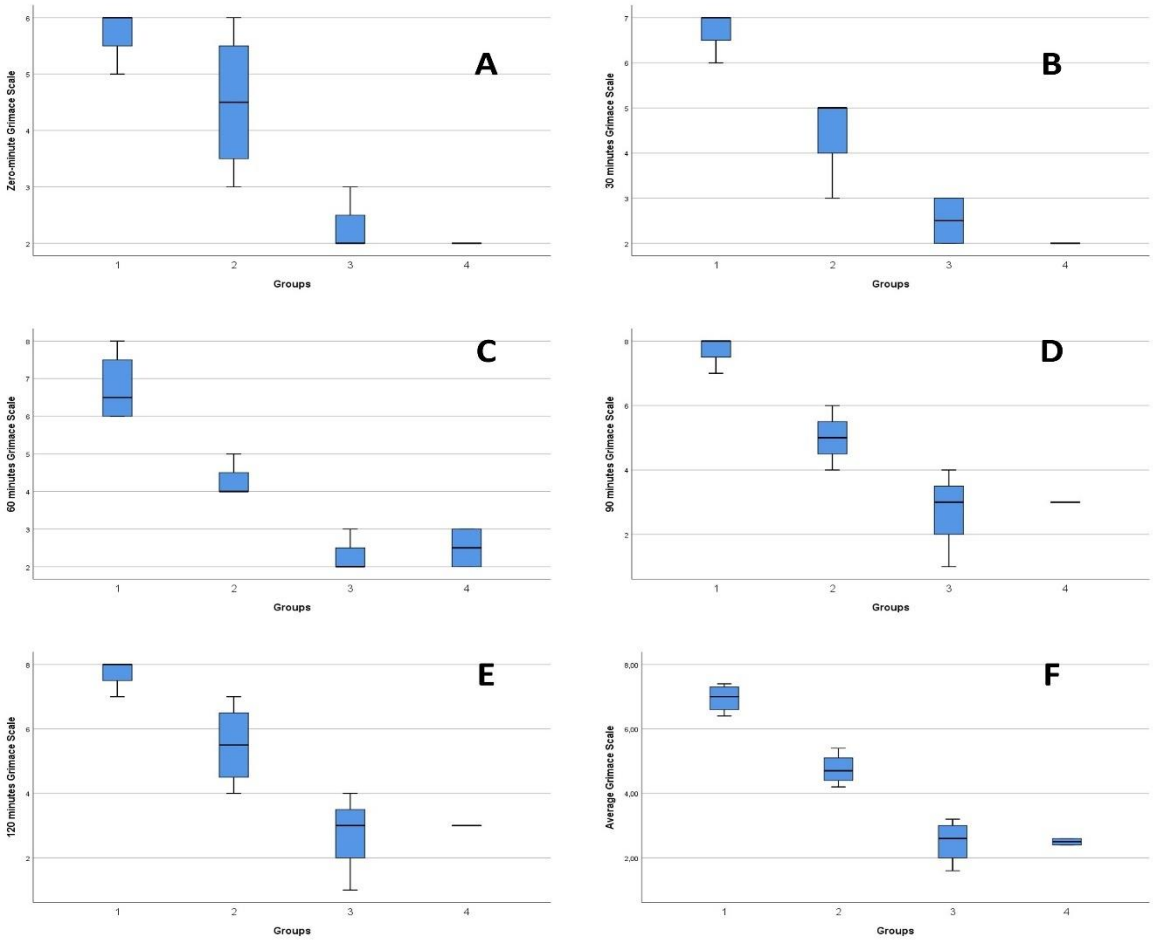

Figure 3. Rat grimace scales (RGS) in groups A: 0-minute RGS scores B: 30-minute RGS scores C: 60-minute RGS scores D: 90-minute RGS scores E: 120-minute RGS scores F: Mean RGS scores in groups

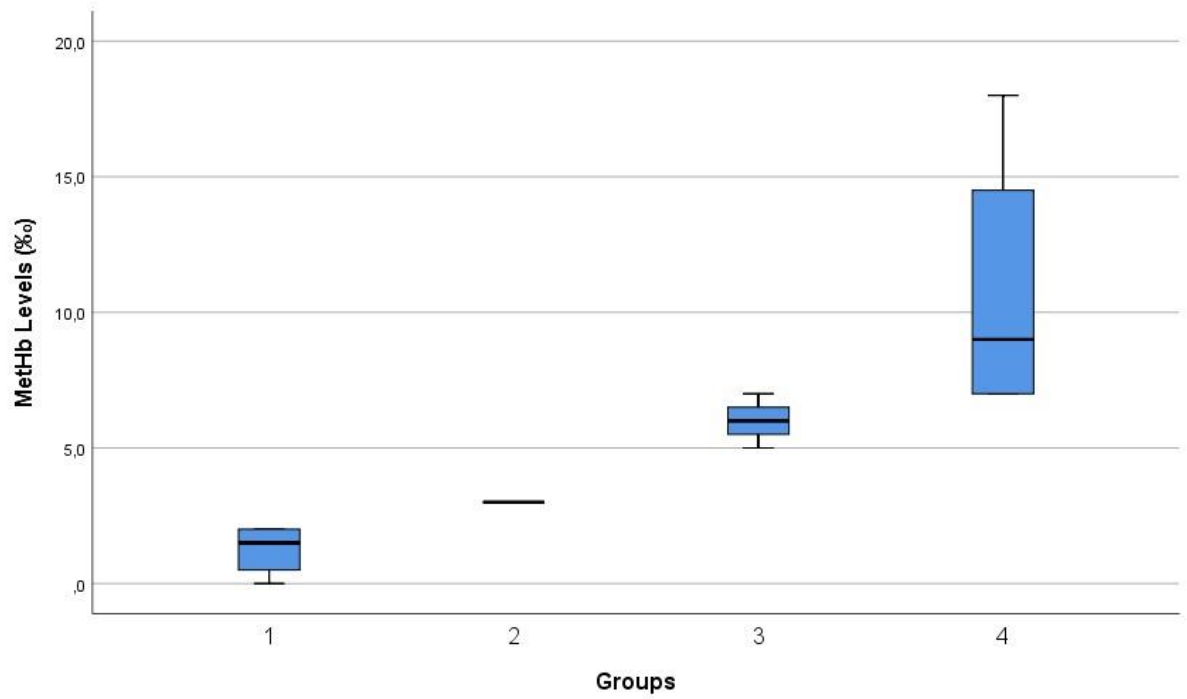

Figure 4. Methemoglobin levels in groups.

Discussion

Numerous studies have been conducted on modeling in animal research in recent decades. Drug research, biochemical markers, and imaging techniques could represent samples through these models (7, 8). These improvements offer the opportunity to research many fields. On top of that, these standardizations were carried to the top with monoclonal experimental animals (9, 10). This study was planned to define the SCALP block model in Sprague-Dawley rats, species that can mimic human physiology significantly.

There are similar studies in the literature which used sedation and muscle relaxants (11-15). On the other hand, some studies used local anesthesia in line with ours (16-19).

Local anesthetic injections to 6 different points in each half of the skull and SCALP block are widely used for many procedures (20). Various studies have been conducted on SCALP block's postoperative pain control and neuroprotective and anti-inflammatory effects (21,22). Chen et al. evaluated the effects of SCALP block on postoperative pain control and the effects of

SCALP block in a meta-analysis including 12 studies (21). These studies showed that SCALP block maintained intraoperative hemodynamic stability (22).

The rat grimace scale is developed to assess pain severity in rats (6,23). Apart from this, different tests such as swallowing analysis, cage monitoring, and nesting have also been defined in rodents (24). In our study, we used the RGS to evaluate the rats in 30-minute periods and assess the pain scale through components such as orbital tightening, nose/cheek flattening, and ear and whisker changes. There are also studies on the use of RGS in anesthesia (25). In their study, Miller et al. used this scale to evaluate general anesthetic agents' effects (25). The SCALP block model described in our study also has the potential to allow the evaluation of local and systemic effects of anesthetic agents in future studies that can be performed by applying the model.

Local anesthetics can act as indirect oxidizers in iron metabolism (26). Therefore, they cause the development of methemoglobin by oxidizing the iron in hemoglobin. Although they are used in local applications, their systemic absorption and side-effect profiles are well-defined (27-30). One of the significant complications of SCALP block is methemoglobinemia. Methemoglobinemia should be considered in clinical situations with low oxygen saturation despite adequate ventilation after local anesthetic admission (26). A blood gas study is sufficient for diagnosing methemoglobinemia; most of the current blood gas devices can analyze the methemoglobin levels. Hence, we analyzed blood gas after the SCALP block to evaluate the presence of methemoglobinemia.

This study had some limitations. First, although the RGS evaluation is a standardized scoring system, the subjectivity of the assessor may affect results. Also, two rats in the very high-dose group died within 30 minutes after the completion of the procedure. Finally, the study did not include long-term results and provided minimal information on whether the SCALP block contributed to late-term postoperative pain control.

Conclusion

The model of SCALP block in rats was defined for the first time in this study, and this article is a pioneer for future studies. However, further work is needed to develop and refine this model.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This study is unfunded.

Ethical approval

The Ankara NeSa Animal Research Laboratory Ethics Committee reviewed the research protocol, and the ethics committee approval was obtained (10.01.2023/003).

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