Transversus abdominis plane block in rats

Transversus Abdominis Plane blocks in rats: Preliminary cadaveric studies.

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Abstract

The transversus abdominis plane (TAP) block is an ultrasound guided regional anaesthetic technique used to provide analgesia to the abdominal wall. Research in humans and cats has demonstrated that TAP blocks reduce pain and post-operative opioid requirements after abdominal surgery. To date TAP blocks have not been described in rats. The optimal technique to employ when performing TAP blocks is controversial with single point injection techniques failing to reliably provide adequate

coverage of the cranial abdominal wall. It has been suggested that performing a two point injection may provide more reliable coverage of the cranial abdominal wall.

The objective of this study was to determine the feasibility of performing ultrasound guided TAP blocks in rat cadavers and to evaluate whether performing a two point technique provides greater spread of injectate than a one point technique when administering the same total volume of methylene blue solution.

Twenty three, four month old, female Sprague Dawley rat cadavers weighing 506±78 grams were used. Transversus abdominis plane blocks were performed using a total of 1mL/kg of methylene blue solution.

Overall success rates for injections were 21.7% (13.6% - 32.8%). Single point injection area of spread was 87.8±32 mm^2 compared to 102.4±17 mm^2 for the two point injection technique.

Due to the low success rate the use of TAP blocks using the current technique cannot be recommended. Two point injection technique appears to provide greater spread, however additional data is required to draw meaningful conclusions

Introduction

Acute pain has been shown to have a multitude of negative effects which may impair research outcomes as well as animal wellbeing. These negative effects may include impairment of sleep, decreased ability to perform physical tasks, and an overall decrease in quality of life [1]. Pain also produces a stress response which may lead to multiple physiologic changes including tachycardia, increased oxygen consumption, tachypnoea, increased susceptibility to infection and hyperglycaemia as well as causing anxiety and depression [2]. Importantly, multiple studies have shown that increasing severity of pain increases the risk for development of chronic pain conditions [3, 4].

The question of which analgesics are most appropriate to employ for specific procedures and types of pain, as well as the optimal dose rate, and frequency of drug administration in rats, remains open. A study assessing the use of analgesics in

research rodents reported worrying rates of analgesic use with 46% of rats undergoing painful surgical procedures not reported as receiving analgesic agents, while only 21% of rats were recorded as receiving analgesics that were not administered for sedative or anaesthetic purposes[5]. In addition to these findings, this paper reported that out of 172 papers describing painful surgical interventions, none reported the use of multimodal analgesic techniques, and no articles described the use of targeted regional analgesic techniques [5]. Both these techniques are considered standard practice in current clinical veterinary medicine [6].

Materials and Methods

Ethical approval was obtained from the University of Edinburgh Veterinary Ethics Committee (VERC 59.20) following development of the protocol. Cadaveric studies using other species were used to determine sample sizes [7-9]. Twenty three freshly frozen 4 month old, entire female rat cadavers weighing approximately 500 grams were obtained after being euthanized as part of a different research protocol, AEC:1852. Rat cadavers were thawed at room temperature for a minimum of twenty four hours prior to use. None of the selected animals had undergone interventions affecting the abdomen prior to enrolment in this study. All imaging and injections were performed by a single investigator trained and experienced in ultrasound guided local anaesthetic techniques. Animals were weighed and marked for identification prior to any interventions. Animals were then placed in dorsal recumbency and the abdomen shaved from the level of the last rib to the pubis. A random number generator (www.random.org, Dublin, Ireland) was used to determine the side of the abdomen to be injected first. The first injection performed was always a single point block, this was followed by the two point block on the contralateral side.

A 15-8 MHz linear ultrasound (US) transducer attached to an ultrasound machine (Sonosite, Fujifilm, WA, USA) was placed in a transverse orientation approximately mid-way between the last rib and the level of the wing of the ileum (Figure 1). The ultrasound image was optimised by using the zoom in function, and the three layers of the abdominal wall were visualised and identified from outer to inner layers as the external abdominal oblique, internal abdominal oblique and the transversus abdominis muscle (Figure 2). A 22G, 2.5 inch spinal needle (BD Medical, Australia)

was inserted using an in-plane technique until the needle tip could be visualised at the level of the fascial plane between the internal abdominal oblique and the transversus abdominis muscle. A small test dose of approximately 0.1ml methylene blue solution was then injected to confirm placement. If the injection was found to be in the incorrect area the needle was readjusted prior to administering another small test dose. The remainder of the volume was then administered for a total of 1 mlLKkg once the needle was visualised as being in the correct place (Figure 3). The ultrasound probe was then placed on the opposite side of the abdomen just below the level of the last rib, and then just cranial to the hip and the procedure was repeated for the two point block, injecting 0.5 mL/kg total per injection site (1 mL/kg total volume). **[insert figure 1]**

Figure 1. Rat cadaver placed in dorsal recumbency. The line on the left of the image shows the injection site for the one point TAP block. The two lines on the right show the cranial and caudal injection sites for two point TAP blocks.

[insert figure 2]

Figure 2. Ultrasound image of a rat cadaver abdomen with the left of the image located towards the midline of the abdomen and the right of the image the lateral aspect of the abdomen. The needle is inserted from the lateral aspect and positioned at the level of the TAP. The top of the image represents the skin surface.

[insert figure 3]

Figure 3. Ultrasound image of successful TAP block. Note the Ventral displacement of the External Oblique & Internal Oblique and dorsal displacement of the Transversus Abdominis Muscle caused by the injectate which appears as a hypoechoic area not present on the previous image

Dissection was performed between five and fifteen minutes following all injections. A midline incision was made using a number 11 scalpel blade and the three muscle

layers were inspected to determine the location of injection (Figure 4). The abdomen was then explored for evidence of intra-abdominal injection. Photos of the spread of injectate were taken against a 30 cm ruler to provide scale for further evaluation using imaging software to determine the size of the area of spread. Images of all injection sites were analysed using ImageJ® software (National Institutes of Health, Wisconsin, USA) to measure the visible area of spread for each block and the area of injectate spread was calculated in mm^2.

[insert figure 4]

Figure 4. Photo of dissected rat cadaver abdominal wall following two point TAP block.

Data was entered into Microsoft Excel® and tidied. Descriptive statistics including mean and standard deviation were calculated for age and weight. Mean, standard deviation and 95% confidence intervals were calculated for spread of injectate. Success rates with 95% confidence intervals were calculated for each injection technique. The location of injectate was recorded and calculated as an overall percentage of all injections to determine the number of successful injections as well as the number of injections made intramuscularly or intraperitoneally. Success rates were then calculated for the first 15 injections and compared to the subsequent injections to identify if a learning curve affected overall success rates. Based on these results a power calculation was performed to determine the additional number of successful injections that would be required to provide statistically significant results.

Continuous data is presented as mean ± SD, proportions are presented in percentage (95% confidence interval).

Results

Mean and standard deviation weight for the rat cadavers used in the study was 506±78 grams.

The three layers of the abdominal wall were able to be visualized and the transversus abdominis plane identified in all 23 cadavers. Overall, 5 out of 23 single point injections, 5 out of 23 cranial two point injections and 5 out of 23 caudal two point injections were successfully injected into the correct fascial plane accounting for a 21.7% (13.6% - 32.8%) success rate for all injection attempts. The success rate for right sided vs left sided injections were similar with 8/69 [11.6% (5.9% - 21.2%)] successful injections on the right side, and 7/69 [10.1% (5.0% - 19.5%)] injections on the left side of the abdomen. Injection success rate for the first 15 injections was 20.3% (12.5% - 31.2%) while the following 15 injections recorded a success rate of 13.0% (7.0% - 23.0%).

Location of injectate was recorded for each injection with 21.7% (13.6% - 32.8%) of injections being deposited purely into the TAP, 21.7% (13.6% - 32.8%) being observed in both the TAP and muscle, 27.5% (18.4% - 39.0%) of injections being deposited into the abdominal cavity, 2.9% (0.8% - 9.9%) being deposited into muscle and the abdominal cavity and 26.1% (17.2% - 37.5%) injected into muscle. Injectate could not be located in 1.4% (0.3% - 7.8%) of injections.

The spread of injectate for the successful one point blocks was 87.8±32.7 mm^2 while the spread for the successful two point blocks was 102.4±17.6 mm^2. Based on these results a power calculation was performed which revealed a further 14 rats with all injections performed being successful would be required to generate statistically significant results.

	One point block	Two point block
Success rate (per injection)	21.7%	21.7%
Success rate of technique	21.7%	13%
Area of spread	87.8 ± 32.7 mm^2	102.4 ± 17.6 mm^2

Table 1. Comparison of results for one point and two point techniques. Per injection refers to a single needle insertion.

Discussion

The aim of this study was to investigate the viability of performing TAP blocks in rat cadavers, while also evaluating the hypothesis that performing two point injection techniques would provide a greater area of injectate spread than one point injection techniques. In the present study only 21.7% of injections were successful. Two point injection techniques demonstrated a greater area of spread than one point techniques, 102.4±17.6 mm^2 and 87.8±32.7 mm^2 respectively, with more successful injections required to gain statistically significant results.

Cadaveric studies in veterinary species have shown particularly promising results with TAP injections generally being successful in close to 100% of attempts [7-9]. One paper however reported significantly lower success rates with 73% of injections deposited purely into the TAP, 23% combined intramuscular and TAP injections in and intraperitoneal injections in 4% of cases [10]. These results may be explained by the relative inexperience of the investigator performing the technique who was a resident in training.

In contrast to this, results from our study have demonstrated significantly greater failure rates with only 21.7% of injections successfully injecting dye into to the TAP, 23% injected into both the muscle and the TAP, and 31% of injections being deposited intraperitoneally.

Multiple factors may have combined in our study to reduce the success rate in these rats.

In this study all injections were performed by one investigator. While it is possible that the low success rate may be attributable to the person performing the injections, it is unlikely that this is the case. The investigator chosen to perform the technique for this study has received formal training in ultrasound guided regional anaesthetic techniques and has been successfully performing this technique in clinical small animal cases for several years. Despite this, performing injections in significantly

smaller animals undoubtedly involves a learning curve as the smaller anatomy is adjusted to and any necessary adjustments in technique are developed.

Learning curves for ultrasound guided blocks have been reported as being particularly steep, with the curve for residents learning to perform brachial plexus nerve blocks plateauing after 10 to 15 attempts [11]. Therefore, it may be assumed that the presence of a learning curve in this study may have negatively affected the results. The effect of this learning curve however appears to be insignificant in this case, as the recorded success rate was similar between the first and second group of 15 injections.

The rats' small size in this study may have served to make identification of important landmarks and the TAP itself more difficult. However, using this ultrasound probe and the zoom-in function, the three muscle layers and the TAP were adequately visualized in all cases. While the operator rated all images as adequate for injection it is possible that the use of a higher frequency probe, and the higher resolution picture provided by this probe, may have aided in the identification and guidance of the needle, which may have improved the overall success rate.

It is likely that the small size of the TAP in these rats, approximately 0.5 mm, and the needles used to perform the injections have contributed to the low success rate. In this study the instance of combined TAP and intramuscular injection, that is injectate observed both in the TAP and intramuscularly after a single injection was 27.5%. This is likely due to the relatively large bevel of commonly used needles when compared to the size of the TAP space in rats. Considering this, it appears likely that even successful injections may deposit some of the injectate intramuscularly, making it harder to predict the total amount of injectate delivered into the TAP space. This may cause more variability in spread of injectate in this model as the actual amount injected into the TAP is likely to vary between animals. The availability of specifically designed needles with smaller bevel lengths may be required to approach success rates reported in other species.

Commented [CB1]: Please see explanation in response letter regarding this point.

The use of Tuohy needles has been previously described in successful TAP blocks in two chinchillas [12]. In this report, with the animals in lateral recumbency, and an 18 MHz linear ultrasound probe was used to visualize the three abdominal muscle

layers, a 22G Tuohy needle was then used to perform the injections. Tuohy needles were originally designed in 1945 for use in epidural anaesthesia. Like Quinke needles, these needles have a relatively long bevel compared to the TAP width in rats. However, unlike Quinke needles they have a curved, blunt tip that ends with the needle orifice ~~(Figure 5)~~. Originally designed to minimize the risk of dural puncture, these needles provide more tactile feedback when passing through tissue planes [13]. In addition to this, the shaft is marked in centimeter gradients which, combined with ultrasound depth markings may help users to confirm they are at the desired depth prior to performing injections. The blunt curved tip combined with the centimeter markings may make it a suitable option for this technique as the curved tip may be more likely to remain entirely within the TAP in rats. Additionally, the extra level of tactile feedback provided by the needle may help the user identify when they are passing through the different muscle planes as has been described in the original landmark based injection studies performed in humans [14].

As mentioned previously, veterinary cadaveric studies have reported success rates which mostly approach 100%. While this study demonstrated significantly lower success rates, more worrying is the number of injections that resulted in injectate entering the abdominal cavity. In this study 31% of injections had injectate noted in the abdominal cavity on inspection compared with other veterinary cadaveric studies in which the presence of injectate in the abdomen following injection is generally a rare occurrence.

To date complications from TAP blocks have not been reported in the veterinary literature. In the human literature, trauma of the liver with associated minor hemorrhage has been reported when only using anatomical landmarks [15], while liver laceration and subsequent septic peritonitis requiring blood transfusion has been reported when performing ultrasound guided TAP blocks [16]. Considering the small size of rats and the ease at which they may be moved while performing injections, coupled with multiple injection locations required for the two point TAP block it is feasible that trauma to a range of abdominal organs may be possible with this technique. Associated complications, as in the human literature, may have minor to major consequences for the animal. Considering the high rate of intraabdominal, and

intramuscular injection the risk of complications using this technique in rats is likely to be significantly higher than has been reported in the human literature.

These finding may be particularly relevant to other small animals, such as chinchillas that have been reported as receiving TAP blocks as part of their clinical treatment. While successful injection was noted on ultrasound evaluation in these cases, thorough intraabdominal evaluation was not performed. The possibility that at least partial peritoneal puncture may have occurred, as in our study, should be considered. Considering the large percentage of animals in this study that received intramuscular or intraperitoneal injections, it would be highly recommendable to perform cadaveric studies on these animals to assess for potential complications.

Unfortunately, in this study there were not enough successful injections to allow investigators to draw any conclusions as to whether two point injections provide greater spread of injectate than one point injections when delivering a total volume of 1ml/kg. Our results showed that the two successful two point injections appeared to provide a greater area of spread than the successful one point injections which is in agreeance with other preliminary studies [7, 17], However, a power calculation following these results showed that another 14 rats with completely successful injections would be required to corroborate statistically this difference. When considering whether a one or two point injection is required for abdominal surgery it is important to consider the area undergoing surgical manipulation. For animals undergoing laparotomy for ovariohysterectomy a single point injection technique may be adequate as the area being surgically manipulated is relatively small. This hypothesis is supported by the study performed by Skouropoulou, Lacitignola [18] which demonstrated the efficacy of single point TAP blocks as part of a multimodal analgesic protocol for post-operative pain control in cats undergoing ovariohysterectomy.

This study has multiple limitations. The use of defrosted cadavers may not accurately recreate interactions with live tissues, which may alter the spread of the methylene blue solution used [9]. Additionally, the use of methylene blue solution has been shown to provide greater spread when injected in cadaveric TAP block models than mixtures containing methylene blue and bupivacaine [19]. This may lead to a larger area of spread than may be expected clinically using common local

anaesthetic drugs. The use of defrosted cadavers may also affect image quality, which due to the small size of the animals being used, may be particularly significant in this study [20].

In the current study, five to fifteen minutes was allowed between injection of methylene blue and dissection of cadavers for evaluation of spread of injectate. This may have led to differences in spread between subjects as some cadavers may have had significantly more time for the injectate to spread compared to others. In addition to this, work with human cadavers has demonstrated that methylene blue injectate does not reach the peak of spread for 40 minutes or longer post injection when performing TAP blocks, further supporting the possibility that spread may be artificially decreased In this study [21]. Finally, while the success rate in this study was very low, it is possible that having an investigator who is very experienced in this technique in cats and dogs may have artificially inflated the ~~expected~~ success rate. It is reasonable to expect that success rates may be lower when this technique is being performed by non-experts. ~~when compared to veterinary staff who may be less familiar with this technique~~.

Conclusion

The use of TAP blocks in rats using this technique cannot currently be recommended due to an unacceptably low success rate and the potential risk of complications associated with the high number of intraabdominal injections. Due to this low success rate, conclusions cannot be drawn regarding the hypothesis that two point injection techniques will provide superior spread of injectate when compared to one point injections techniques when using the same total volume of injectate. Further research should be conducted with the aim of increasing success rates.

Declarations

The authors declare no potential conflicts of interest. No funding was received for this study

Study data is available via the corresponding author if requested via email.

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