October 17, 2018

Dear Editor in Chief,

Thank *y*ou for giving us the opportunity to revise and resubmit this manuscript titled **" The effect of clonidine on the cutaneous silent period during spinal anesthesia " (Manuscript NO 42131).** We would like to thank the reviewers for the careful and valuable review of this manuscript. We appreciate their appropriate and constructive suggestions which helped us to improve the quality of this paper. We have addressed all the issues raised and have modified the manuscript accordingly. We have tried to answer all the questions and make corrections as the reviewers suggested. As instructed, the revised elements are in red color of the main text in the manuscript.

Below is a summary of the changes we performed and our response to the reviewers (the reviewer's comments are in *italic*).

We hope that you will accept our comments and answers.

Kindest regards

Miroslav Zupcic, PhD

Responses to the comments from the editorial board member and reveiwers:

1 Reviewer's code: 02484487

SPECIFIC COMMENTS TO AUTHORS

Clonidine has been used to prolong the effect of SAB but this article has measured cutaneous silent period(CSP) and CSP latency durindg block regression after SAB which gives an added information regarding the use of clonidine. Further studies can be done to highlight the effect of clonidine. Effects on MAP and heart rate are not significat but VAS over 24hrs was also significant.In my opinion this is an interesting article wirh the collaboration od neurology faculty.This can be published

Answer:

Thank you for your recommendation.

2 Reviewer's code: 00504975

SPECIFIC COMMENTS TO AUTHORS

This is an interesting study that assessed the effect of adding clonidine to a subarachnoidal block with levobupivacaine on the cutaneous silent period and its latency in patients scheduled to inguinal repair. Not surprisingly, the Authors found that levobupivacaine plus clonidine shortened the CSP and prolonged its latency in comparison to levobupivacaine alone. Although the results from this study could contribute to the field of CSP, I have some concerns with the way that the results were analysed and the manuscript is presented. Please refer to the points below for specific details. Abstract In the Methods section, please include the dose rate of levobupivacaine and clonidine administered to the patients.

Accepted with thanks and corrected.

Answer:

The ammended part of the Abstract now states:

METHODS

A total of 67 adult patients were included in this randomized, prospective, single-centre, double blind trial. They were without neurological disorders and were scheduled for inguinal hernia repair surgery. This trial was registered on ClinicalTrials.gov (NTC03121261). The patients were randomized into two groups with regard to the

intrathecally administered solution, either 0.5% levobupivacaine 15 mg with 0,015% clonidine 50 μg or 0.5% levobupivacaine 15 mg alone [34 patients in the levobupivacaine-clonidine (LC) group and 33 patients in the levobupivacaine (L) group]. CSP and its latency were measured four times: prior to the subarachnoid block (SAB), after motor block regression to Bromage 0 level, of the Bromage scale, with ongoing sensory blockade and 6 and 24 hours after SAB.

In the Results section, you need to mention that only data from 30 patients in each group were analysed.

Accepted with thanks and corrected.
The ammended part of the Abstract now states:

RESULTS

Only data from 30 patients in each group were analysed. There were no significant differences between investigated groups preoperatively and after 24 hours. CSP of the L group at the point of time when the Bromage scale was 0, was 44.8 ms ± 8.1, while in the LC group it measured 40.2 ms ± 3.8, P=0.007. The latency in the L group at the point of time when the Bromage scale was 0, was 130.3 ms ±10.2 and in the LC group it was 144.7 ms ± 8.3, P<0.001.

In the Conclusion section, you need to mention that these observations with levobupivacaine and clonidine were in comparison with levobupivacaine alone.

Accepted with thanks and corrected.
The ammended Conclusion section now states:

CONCLUSION
Intrathecal addition of clonidine to levobupivacaine for SAB in comparison with levobupivacaine alone results in a diminished inhibitory tonus and shortened CSP.

Core tip What is the relevance of the statement "Duration of the CSP and its latency are altered in polyneuropathy and various diseases of the central nervous system" for you study? Consider deleting it.

Accepted with thanks and deleted.
The core tipe now states:

Cutaneous silent period (CSP) is an oligosynaptic spinal inhibitory reflex. The results of our study show that intrathecal administration of levobupivacaine with added clonidine, in comparison to levobupivacaine alone, yields a statistically significant shorter CSP and a significantly longer CSP latency during block regression, after subarachnoid block application. Accordingly, we can conclude that during subarachnoid block regression, a small dose of intrathecally administered clonidine ameliorates the inhibitory tonus and accelerates the conduction in the oligosynaptic spinal circuit.

Introduction Please rephrase the sentence "Clonidine exerts its analgesic effect via Aδ, C-fibres and substantia gelatinosa of the spinal medulla, however, considering that its analgesic effect is the strongest after intrathecal administration, it is deemed that the primary effect site of the action of clonidine is the spinal medulla[11,12]." You are taking about the site of analgesic action for clonidine, but by mentioning 'Clonidine exerts its analgesic effect via' you making sounds as if you were taking about its mechanism of action. Also, please change 'spinal medulla' for 'spinal cord'.

Accepted with thanks and corrected:
The ammended Introduction section now states:

The analgesic effects of clonidine are achieved through the Aδ, C-fibres as well as the substantia gelatinosa of the spinal cord, however, considering that its analgesic effect is the strongest after intrathecal administration, it is deemed that the primary effect site of the action of clonidine is in fact the spinal cord [11,12].

Please rephrase you primary hypothesis. As it reads, it is difficult to understand what you tried to assess. This would be a good place to include your hypotheses; please provide them.

Accepted with thanks and corrected:
The ammended Introduction section now states:

… Animal studies have shown that intrathecal administration of clonidine immediately resulted in a facilitation of the spinal reflexes in spinalized rats [mechanical or functional transection of the spinal cord (spinal block with procaine)] [13]. Until now, the CSP has never been measured during neuraxial intrathecal block or after intrathecal administration of clonidine. We hypothesize that there could be a more pronounced and prolonged effect on the cutaneous silent period after intrathecal administration of levobupivacaine and clonidine compared to levobupivacaine alone.

Materials and methods The M&M section would benefit from deletion of repetitive material (repeating other sections) and could use significant tightening up. A few examples are provided below. - The sentence "The day before surgery, all included patients were referred to the department of neurology where a blinded neurologist conducted a primary measurement of CSP and its latency with an EMG device (Medelec Sinergy, UK)" would be better in the "Cutaneous silent period measurements" section. - The information on levobupivacaine and clonidine is repeated within the same (very long!) paragraph in the "Study flow and anesthesia procedure" section.

Accepted with thanks and corrected.
The repeated information has been significantly ammended and the CSP measurement details have been moved to the "Cutaneous silent period measurements" section.
The ammended " Study flow and anesthesia procedure" section now states:

The patients were randomized through a free online randomization service (Urbaniak, G. C., & Plous, S. (2013). Research Randomizer (Version 4.0) [Computer software]. Retrieved in May, 2017, from http://www.randomizer.org. Eligible patients were randomly allocated to receive either a SAB with levobupivacaine and clonidine [levobupivacaine - clonidine group (LC group, n=34)] or levobupivacaine [levobupivacaine group (L group, n=33)]. The neurologists and anesthesiologists conducting the clinical part of the study were not aware of the randomization numbers of individual patients, which was known only to two anesthesia technicians who were not involved in the selection and follow up of patients. The control (L) group received 0.5% levobupivacaine (Chirocaine®, Abbott Laboratories, Dublin, Ireland) 15 mg with 0,9% saline 0.33 ml and 40% glucose 0.5 ml while the experimental (LC) group received 0.5% levobupivacaine 15 mg with 0,015% clonidine (0.015% Catapressan; Boehringer Ingelheim KG, Germany) 50 µg and 40% glucose 0.5 ml. The CSP and it' s latency were measured one day before surgery in order to avoid an influence of benzodiazepine premedication. All patients received premedication with midazolam (Dormicum®, Roche) 5 mg intramuscularly (IM)...

The repetitive elements from the sentance "At this time point, the CSP was measured by the same neurologist as previously in the EMG laboratory at the department of neurology." We have abbreviated and now states At this time point, the second CSP measurement was conduced in the EMG laboratory.

The ammended "Cutaneous silent period measurements" section now states:

All included patients were referred to the department of neurology where a blinded neurologist conducted a measurement of CSP and its latency with an EMG device (Medelec Sinergy, UK).

Please combine this information. - "…recorded the pain intensity using a visual analogue scale (VAS) scale (0-10, 0 no pain, 10 maximum pain)" would be better in the section "Pain intensity assessment using VAS" – "

Accepted with thanks and corrected:
The sentance in the " Study flow and anesthesia procedure" section " After CSP measurement, the patients were returned to the surgical ward where a ward technician, recorded the pain intensity using a visual analogue scale (VAS) scale (0-10, 0 no pain, 10 maximum pain) and return of sensation to the sacral (S) 1 dermatome using a pinprick test method." Has been abbreviated and now states" After CSP measurement, the patients were returned to the surgical ward where a ward technician, recorded the pain intensity and return of sensation to the sacral (S) 1 dermatome using a pinprick test method. " The information on the visual analogue scale (VAS) has been moved to the "Pain intensity assessment using VAS" section and now states:
Pain intensity was measured using VAS scale (0-10, VAS 0= no pain; VAS 10= maximal pain) prior to the surgical procedure and every three hours thereafter, during the 24-hour postoperative period. The administration time of analgesics was also measured over the same period. In the case of moderate postoperative pain (>3 VAS pain score <6), the patients received ketoprofen (Ketonal, Sandoz) 100 mg IV in 100 mL of 0,9% saline over 15 minutes . In the case of severe postoperative pain (VAS ≥ 6), the patients received tramadol hydrochloride (Tramal®, Herds) 100 mg in 500 mL of 0,9 % saline over 30 minutes[17]. According to previous studies, tramadol significantly prolongs the duration of CSP and these patients were excluded from the study[18].

*and the instructions for software support(G*Power3.1manual,http://www.gpower.hhu.de/fileadmin/redaktion/Fakultaeten/Mathematisch-Naturwissenschaftliche_Fakultaet/Psychologie/AAP/gpower/GPowerManual.pdf).*
*Analysis was carried out with the software support of G*Power for Windows, version 3.1." Please combine this into one sentence.*

Accepted with thanks and corrected:

…Power analysis was carried out with the software support of G*Power for Windows, version 3.1.2" (http://www.gpower.hhu.de/fileadmin/redaktion/Fakultaeten/Mathematisch-Naturwissenschaftliche_Fakultaet/Psychologie/AAP/gpower/GPowerManual.pdf).

Please provide the manufacturer's details for the pulse oximetry finger probe used.

Accepted with thanks and corrected:
In the operating theatre all patients were non-invasively monitored- MAP with Non invasive blood pressure (NIBP) cuffs (Dräger Medical GmbH, Lübeck, Germany), peripheral oxygen saturation (SpO2) and HR via a pulse oximetry finger probe (SpO2 Sensor, Adults, Reusable,Dräger Medical GmbH, Lübeck, Germany).

Please specify how HR and MAP were measured.

Accepted with thanks and corrected:
In the operating theatre all patients were non-invasively monitored- MAP with Non invasive blood pressure (NIBP) cuffs (Dräger Medical GmbH, Lübeck, Germany), peripheral oxygen saturation (SpO2) and HR via a pulse oximetry finger probe (SpO2 Sensor, Adults, Reusable,Dräger Medical GmbH, Lübeck, Germany)..
As previously desribed in the manuscript the MAP and HR were measured every 5 minutes.

"…estimated the motor block regression as Bromage 0 (ability to move the legs at the hip, knee and foot) while the sensory block was still present". As it reads, it seems that the anaesthesiologist scored motor block as zero in the Bromage scale rather than assessed motor block. Please rephrase.

Answer:
In the methodology of this investigation it was important to classify Bromage as O because the second measurement of the CSP and its latency was in the period of motor block regression (i.e. Bromage 0), with an ongoing sensory block. Namely, to achieve a CSP the muscle contraction is of extreme importance so the CSP and its latency had to be measured after motor block regression. On the other hand, our aim was to find out the CSP and latency under the presence of spinal anesthesia, so therefore it had to be measured before sensory block regression.

Please add details of how long the VAS line was. It seems that ketoprofen and tramadol were diluted and given as a constant rate infusion. Please provide details of the rate of infusion for these drugs.

Accepted with thanks and corrected:

Ketonal and Tramadol were administered as a single dose according to the study protocol, depending on the presence of pain, which was measured every three hours and the choice of drug depended on the intensity of pain as per VAS scale. In the case of moderate postoperative pain (>3 VAS pain score <6), the patients received ketoprofen (Ketonal, Sandoz) 100 mg IV in 100 mL of 0,9% saline. In the case of severe postoperative pain (VAS ≥ 6), the patients received tramadol hydrochloride (Tramal®, Herds) 100 mg in 500 mL of 0,9 % saline. In the manuscript, in accordance to your instructions we have better defined the method of administration of the analgesics and the sentence now states:

The administration time of analgesics was also measured over the same period. In the case of moderate postoperative pain (>3 VAS pain score <6), the patients received ketoprofen (Ketonal, Sandoz) 100 mg IV in 100 mL of 0,9% saline over 15 minutes . In the case of severe postoperative pain (VAS ≥ 6), the patients received tramadol hydrochloride (Tramal®, Herds) 100 mg in 500 mL of 0,9 % saline over 30 minutes[17].

As mentioned earlier in the methods section, the VAS was measured prior to the surgical procedure and every three hours thereafter, during the 24-hour postoperative period.

Consider changing "lying supine" by "lying horizontally" or "in the supine position", and "big toe" for "hallux".

Accepted with thanks and corrected:
The ammended "Patients and methods" section now states:

...The patients were lying horizontally and a stimulating ring electrode was placed on the hallux of the leg on the operated side, while a plain surface registering electrode was placed above the ipsilateral tendon of the extensor digitorum brevis muscle.

Please provide details on how "the duration of an individual stimulus was gradually increased to 1 ms." How was this gradual increase carried out, what was the rate of increase?

Accepted with thanks and corrected:
As in the methods of Svilpauskaite et al [21] to obtain a CSP and its latency, we also gradually increased the duration of the individual stimulus/i. Namely, we had increased the stimulus at a rate of 0.1 ms to a total value of 1 ms with the aim of achieving a reproducible duration of CSP and its latency.

The ammended "Patients and methods" section now states:

In the case where EMG silence was not able to be evoked, the duration of an individual stimulus was gradually increased at a rate of 0.1 ms to 1 ms, with the aim of achieving a reproducible duration of CSP and its latency[21].

In "The measurement was repeated up to 10 times in 30 seconds intervals and an arithmetic mean (of) three best measurements (complete EMG silence and longest duration of CSP) was calculated" consider removing the brackets and incorporating the information within them into the sentence.

Accepted with thanks and corrected:

The measurement was repeated up to 10 times in 30 second intervals and an arithmetic mean of three best measurements, which yielded a complete EMG silence, making possible for the longest duration of CSP to be calculated.

The statement "Sample size consideration was made due to the presumption of a previously published similar study…" needs a reference.

Accepted with thanks and corrected:
Sample size consideration was made due to the presumption of a previously published similar study[9].

In "Quantitative values are shown through", please change "through" by "as". It is not clear what comparisons were made with the different statistical analyses described in your manuscript. Please reword this section and make sure you have used appropriate statistical tests for your study.
From what it is described in the "Statistical analysis" section and information at the bottom of Table 2, it seems that you used a two-way RM-ANOVA and various t-tests to analyse your data on CSP and CSP latency. For this a two-way RM-ANOVA followed by Bonferroni correction should have been appropriate. Why did you report that RM-ANOVA was used and then various t-tests performed?

Also, "All relevant data from RM-ANOVA analyses and least squares means (LS-means) with standard errors (SE) were computed for each effect." Why was this done? What is the clinical relevance of it? Wouldn't suffice with analysing the individual data points specified in the study? Alternatively, if the overall effect across time would be of interest, AUC values should have been computed for each variable and AUC values analysed. By only analysing the means and not considering time, this analysis seems irrelevant.

Thank you for your comment. It has been changed according to suggestion.
We removed mark „Tested with RM-ANOVA" since results in these table do not represent RM-ANOVA. We used independent t-test to assess differences between investigated groups and dependent t-test to analyse differences before and after operation.
Bonferroni correction for adjustment for multiplicity has been applied to overall and time-specific treatment differences.

The ammended "Statistical analysis" section now states:

Quantitative values are shown as the means, standard deviation and 95% confidence interval (95% CI). Kolmogorov-Smirnov test was used to analyze the distribution of quantitative data and according to the data obtained we applied appropriate parametric tests. The comparisons between the quantitative values were made with the independent and dependent t-test. The differences in categorical values were analyzed with Fisher's exact test. A two-way repeated measured analysis of variance (RM-ANOVA) was conducted to evaluate the null hypothesis that there are no changes in CSP, latency of CSP, VAS score and secondary hemodynamic parameters (MAP, HR) before, during and after spinal anesthesia, (time and treatment over time interaction). Overall and time-specific treatment differences were generated with adjustment for multiplicity – Bonferroni correction. All other P values less than 0.05 were considered significant. IBM SPSS Statistics, version 21.0 was used in the analysis. (www.spss.com).

Results Please rephrase the first sentence. It was somehow mentioned in M&M that 67 patients were enrolled (although it should be better stated) and there is no need to repeat it in the Results. Mention here that 4 and 3 patients from the LC and L groups, respectively, were excluded from the trial and that only 30 patients per group were included in the analysis.

Accepted with thanks and corrected:
The ammended Results now states:

4 patients from LC group and 3 patients from the L group were excluded from the trial due to discomfort during performance of the CSP test and only 30 patients per group were included in the analysis...

There is no need to include Figure 1; consider deleting it.

Accepted with thanks and deleted

There is mention of a "Mann Whitney U test" here, but not in the "Statistical analysis" section. Please amend Consider deleting "Data regarding CSP and latency of CSP before, during and after spinal analgesia are shown in Table 2.", it is not needed.

Accepted with thanks and corrected:

4 patients from the LC group and 3 from the L group were excluded from the trial due to discomfort during performance of the CSP test and only 30 patients per group were included in the analysis. Data about patient characteristics and duration of surgery, did not show statistically significant differences between groups (Table 1).

In the sentence "There were no significant differences between investigated groups preoperatively and after 24 hours" you need to specify what parameters were not significantly different.

Accepted with thanks and corected:

There were no significant differences in CSP and it's latency between investigated groups preoperatively and after 24 hours.

The second paragraph in the "Results" section is too long and mainly repeats what is summarised in Tables 2, 3 and 4. This whole paragraph needs to be reworded while omitting repeating exactly what it can be seen in Tables 2, 3 and 4. Alternatively, these tables could be deleted although I believe that these types of results are easier to read, compare, and contrast from a Table than from the main text.

Accepted with thanks and corected

Similarly, Figure 2 is not needed as it repeats the same information already presented in Table 2.

Answer:

We have decided to keep Figure 2 (in the revised manuscript it is Figure 1) considering the beneficial clarity of displayed dynamic changes in the CSP and its respective latency

Again, the "Summarized results from repeated measures ANOVA" seem irrelevant and should be omitted.

Accepted with thanks and omitted

Discussion As for the M&M section, the Discussion, particularly the section on CSP and CSP latency, could use significant tightening up. In "The results of the first measurement of CSP and latency were similar between groups, which is suggestive of adequate patient selection", "randomisation" rather than "selection" would describe this situation. Please delete "CSP of the L group in the first measurement in this study was 62.1 ms ± 9.5, while in the LC group measured 61.3 ms ± 6.2 . The latency in the L group in the first measurement was 108.2 ms ± 11.6, and in the LC group was 107.2 ms ± 11.6." These are results and do not add anything to your discussion.

Accepted with thanks and corrected.

The ammended Discussion section now states:

The results of the first measurement of CSP and latency were similar between groups, which is suggestive of adequate patient randomisation.

The sentance "CSP of the L group in the first measurement in this study was 62.1 ms ± 9.5, while in the LC group measured 61.3 ms ± 6.2 . The latency in the L group in the first measurement was 108.2 ms ± 11.6, and in the LC group was 107.2 ms ± 11.6." has been deleted.

"Until now, the CSP has never been measured during neuraxial intrathecal block or after intrathecal administration of clonidine." Was this something that motivated the study? If so, it should be included in the Introduction.

Accepted with thanks and corrected:

Sentence „Until now, the CSP has never been measured during neuraxial intrathecal block or after intrathecal administration of clonidine." Has been removed from discussion and has been included in the Introduction.

Research results Again, what is the relevance of reporting "24-hour period" results when only mean values were considered and time was obviated at all?

Accepted with thanks and corrected.

Research conclusion Please replace "local anesthetics" by "levobupivacaine".

Accepted with thanks and corrected.

References Ref. 9. Journal title should be "Essays" not "Esseys".

Accepted with thanks

Ref. 10. Journal title should be "Anaesthesiol" not "Anesthesiol".

Accepted with thanks

Ref. 22. Manuscript title should be in "Sentence case" not "Title Case".

Accepted with thanks

Figures Figure 1 and 2 are not needed. Consider deleting them.

Answer:
Figure 1 has been deleted while we have decided to keep Figure 2 (in the revised manuscript it is Figure 1) considering the beneficial clarity in the displayed dynamics of the changes in CSP and its latency

Figure 3 and 4. Please provide enough information in the figure legends so that the readers can fully understand what was done without the need to refer back to the main text.

Accepted with thanks and corrected:

Tables As for figure legends, please provide enough information in the table titles legends so that the readers can fully understand what was done without the need to refer back to the main text.

Accepted with thanks and corrected:

Table 1. Although intuitive that for gender the remainder of participants were female, it is not possible to know without referring to the main text that the ASAPS for the remainder of the patients was 1. Please include these data in the table.

Accepted with thanks and corrected.

Table 2. The repetitive use of "Mean ± SD" is not needed. Please include this information in the table title. Also, please report meaningful comparisons. These should be within group across time and between groups at the same time point. For this RM-ANOVA followed by Bonferroni correction should have been used. Why did you report that RM-ANOVA was used and then various t-tests?

Accepted with thanks and corrected.
Thank you for your comment. As we have said before It has been changed according to suggestion. We removed mark „Tested with RM-ANOVA" since results in these table do not represent RM-ANOVA. We used independent t-test to assess differences between investigated groups and dependent t-test to analyse differences before and after operation.

Table 3. As for Table 2, the repetitive use of "Mean ± SD" is not needed. Please include this information in the table title.

Accepted with thanks and corrected.

Table 4. As explained before, these results seem irrelevant and do not represent what happened during the 24 hours of assessments in this study. Please delete this table.

Accepted with thanks and deleted