ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

The morphofunctional impact of topical Lidocaine formulation in inflammatory pain – experimental study

MARIA-MAGDALENA LEON-CONSTANTIN[1)], TEODORA ALEXA-STRATULAT[2)], ANDREI LUCA[3)], BOGDAN IONEL TAMBA[3)], LAURA MIHAELA TRANDAFIR[4)], VALERIA HARABAGIU[5)], ELENA COJOCARU[6)]

[1)] *1st Medical Department, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[2)] *Department of Medical Oncology–Radiotherapy, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[3)] *CEMEX Laboratory, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[4)] *Department of Mother and Child – Pediatrics, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[5)] *"Petru Poni" Institute of Macromolecular Chemistry, Iaşi, Romania*

[6)] *Department of Morphofunctional Sciences I – Pathology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

Abstract

Background: Despite all the technological progresses and conventional medical treatments, pain therapy remains a challenge for both children and adult patients. *Objective*: The aim of the study was to identify new transdermal Lidocaine delivery systems that will ensure a controlled release, an improved availability and will finally enhance local pain control. *Materials and Methods*: The experimental animals were adult Wistar rats weighing between 180–200 g. At the beginning of the experiments, we induced an inflammatory pain by administering λ-carrageenan, using a previously known model. Subsequently, the animals were subjected to a battery of nociception tests: algesimeter, cold plate and hot plate. Once the paw edema was obtained, the tested substance containing Lidocaine or eutectic mixture of local anesthetics (EMLA) cream was applied on the hind paw. Since we wanted to identify if our substance causes any local effect, the samples were collected from the paw, from the area where it was administered. *Results*: At algesimetric testing, it has been showed that the tested substance enters into its action slowly, up to 15 minutes after administration and reaches it maximum effect within 15–30 minutes, then the effect diminishes but does not disappear even at two hours. When compared to EMLA, the analgesic cream that exists in the markets, our substance has superior analgesic properties at 15 and respectively 30 minutes after administration. The effect of the tested substance is still present after two hours, lower than in the maximum range (15–30 minutes), but higher than EMLA's effect. The histopathological exam revealed an inflammatory reaction in all groups. *Conclusions*: The tested substance proved analgesic properties longer and higher than EMLA but did not influenced the inflammatory reaction.

Keywords: Lidocaine compound, pain, EMLA, inflammation.

Introduction

In the face of all technological developments and well-known medical treatments, pain management in pediatric and adult patients continues to be a poorly elucidated problem worldwide [1, 2], considering the three features involved in its pathogenesis: nociception [3, 4], emotional factors [5, 6], and behavioral factors [7, 8]. Specialists are currently facing difficulties in pain control, such as cognitive impairment of drugs due to central effects, partial efficiency of analgesics, and systemic effects, which limits the therapeutic outcomes [9–11].

The essential target of topical preparations development is the improvement of patient compliance to medical treatment, by providing efficient pain relief with less central nervous system effects and minimal drug regimen burden [12–14].

The aim of the study was to identify new transdermal Lidocaine delivery systems that will ensure a controlled release, an improved availability and will finally enhance local pain control. Transdermal drug penetration depends on several factors, especially on the delivery system used for the hydrophobic active drug.

Materials and Methods

Animals

Male adult Wistar rats ("Victor Babeş" National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania), weighing between 180–200 g were used. The animals were kept in a room with controlled temperature (21±2°C), with circadian rhythm 12 hours light/12 hours dark, and were allowed to accommodate in this climate for at least 24 hours before the experiment. There was one rat per cage, with free access to food and water. The cages used were standardized polycarbonate (Sapaco) cages, measuring 1500 cm^2.

The study was performed after obtaining the approval from the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania, and after receiving the testing chemical compound from the "Petru Poni" Institute of Macromolecular Chemistry, Iaşi. The study was carried out in accordance with the recommendations of the European Community (EC) regarding the use of medicinal products in preclinical studies (Regulation of the Council of the EC No. 86/609/EEC

from 24th of November 1986), in conformity with the conventions of good laboratory practice, published in the Official Gazette, Part I No. 102 from 06/02/2002 (approved by the Government of Romania, Decision No. 63 from 24th of January, 2002), as well as in accordance with clinical norms and protocols regarding the testing of drugs (approved by the Ministry of Public Health No. 906 from 25th of July, 2006). In addition, nociceptive tests were performed, meeting the standards recommended by the Ethics Committee of the International Association for the Study of Pain (IASP): the recommendations of the "Declaration of Helsinki"; "Guiding Principles in the Care and Use of Animals" approved by the *American Physiological Society* (published in *Journal of Neurophysiology*); and "Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals" [15–17].

Environmental impact

The biological products and the disposable materials that came in contact with it were removed according to the current Guidelines. The activities carried out within the laboratory did not raise other problems of environmental protection.

Methods

The rats were divided in three groups: group C (λ-carrageenan), group E+C [eutectic mixture of local anesthetics (EMLA) and λ-carrageenan] and group T+C (tested substance and λ-carrageenan). Each group included 10 animals: two controls and eight experimental animals. Inflammatory hyperalgesia was induced at the beginning of the experiment, by a λ-carrageenan injection. Once the paw edema was obtained, the tested substance containing Lidocaine or EMLA was applied on the hind paw. Subsequently, the rats were subjected to a battery of nociception tests: algesimeter, cold plate and hot plate. The assessments were performed at specified intervals (5, 15, 30 minutes, one hour, two hours). In order to evaluate the antinociceptive effect of these two substances, the paw withdrawal thresholds (PWTs) were determined for mechanical (algesimeter) and thermal (cold plate and hot plate) stimuli. It was decided to perform the tests after the animal's hair removal.

Euthanasia of laboratory animals

At the end of the experiment, the animals were euthanized, without any physical or mental suffering, according to the *American Veterinary Medical Association* (AVMA) Protocol of Euthanasia from 1993 (the method must be painless, to rapidly produce a state of unconsciousness, cardiac arrest, respiratory arrest and death). This is usually a standard procedure, performed in special necropsy rooms, separated from the place where other animals are kept.

The inhalation of a volatile anesthetic (Isoflurane) induced the unconsciousness within few seconds. The absence of vital signs (heartbeats, respiratory movements, reflexes) followed almost 5 minutes after. After confirming the death, tissue samples were collected.

The following drugs were used in the experiment: λ-carrageenan, which is a high molecular weight sulphated polysaccharide obtained from red algae with inflammatory properties; the tested substance, which was synthesized by "Petru Poni" Institute and delivered as a powder, which is soluble in saline solution; it is formed by a mixture of polymers and Lidocaine, which facilitate penetration of drug in the skin; EMLA – a eutectic mixture of 2.5% Lidocaine and 2.5% Prilocaine formulated as an oil-in-water emulsion, known for its analgesic properties [18].

Histopathological assessment

Since we wanted to identify if the substance causes any local effect, the samples were collected from the paw, from the area where the substance was administered. The collection of samples was performed after euthanasia of animals and were specifically processed, by paraffin inclusion, microtome sectioning (3–5 μm), and usual stainings – Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichrome [19, 20].

Data analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical assessment was performed by means of Statistical Package for the Social Sciences (SPSS) *v.*20 and GraphPad Prism 6.0 software. Repeated measures analysis of variance (ANOVA) was used to assess time and substance effect. The significance level was set *a priori* at $p<0.05$.

Results

Pharmacological testing of the researched substance

The assessments of the algesimetry indicated that the effect was installed at 15 minutes after administration (at 5 minutes none of the substances had effects). At 15 minutes after administration, a superior effect of tested substance was observed compared to EMLA cream (2.5% Lidocaine and 2.5% Prilocaine). The effect was still present after 30 minutes (statistically significant), but ceased before the end of the experiment (Figure 1).

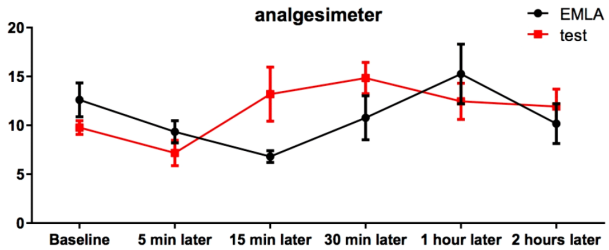


Figure 1 – ***Algesimeter testing of the researched substance vs. EMLA. EMLA: Eutectic mixture of local anesthetics.***

At one hour, the effect of tested substance was less powerful than EMLA's, but became higher at the two-hours testing, being almost the same as the effect obtained at 15 minutes after administration. Therefore, the onset of action after administration was within 15 minutes, the peak effect occurs within the range 15–30 minutes, then the effect diminishes, but does not disappear neither at two hours. The results obtained with the algesimeter were further confirmed by the cold plate test, which showed

that the therapeutic effect of the tested substance was significantly higher than that of EMLA at 15 minutes, then it starts decreasing becoming after one hour similar to EMLA's. The peak effect was at 30 minutes after administration, when using the cold plate test (Figure 2).

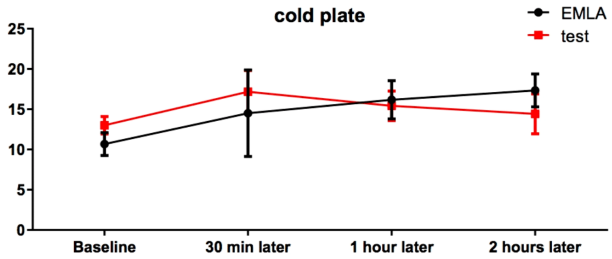


Figure 2 – ***Cold plate testing of the researched substance vs. EMLA. EMLA: Eutectic mixture of local anesthetics.***

When we performed the hot plate test, the measurements showed that the tested substance had a more powerful effect at the 5, 15 and 30 minutes evaluations. As in the previous tests, the effects of the two substances were similar at the one-hour testing. The effect of our tested compound decreased after two hours (Figure 3).

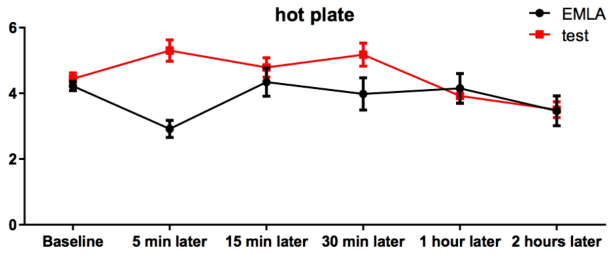


Figure 3 – ***Hot plate testing of the researched substance vs. EMLA. EMLA: Eutectic mixture of local anesthetics.***

As a conclusion of the performed tests, the action of the tested substance settles within 15 minutes, reaches the peak effect at 30 minutes and then falls of, persisting at a low intensity at the last testing, at two hours after administration.

Results of the histopathological assessment

In both HE and GS trichrome stainings, skin samples collected from the control animals revealed a normal architecture: epidermis (thin keratinized stratified squamo-cellular epithelium), superficial dermis (loose connective tissue), and deep dermis (dense connective tissue). The pilosebaceous, apocrine, and eccrine glands were free of pathological changes (Figure 4, a–c).

The skin samples collected from the group C were suggestive for a mild acute inflammatory reaction: rare inflammatory elements [polymorphonuclear neutrophils (PMN)], discrete edema in the superficial and deep dermis and mild vascular congestion (Figure 5, a and b).

The skin samples collected from the group E+C revealed changes suggestive for a more intense acute inflammatory reaction. The inflammatory elements were represented by PMN, with diffuse disposition in the superficial and deep dermis, abundant in the deep dermis (the place of injection), with perivascular disposition. Edema and vascular congestion with leukocytosis were also observed (Figure 6, a–c).

For the group T+C, the following elements were identified: frequent PMN, especially in the deep dermis, with perivascular and perineural dispersion, vascular congestion with leukocytosis, interstitial edema and focal areas of microhemorrhages (Figure 7, a–d).

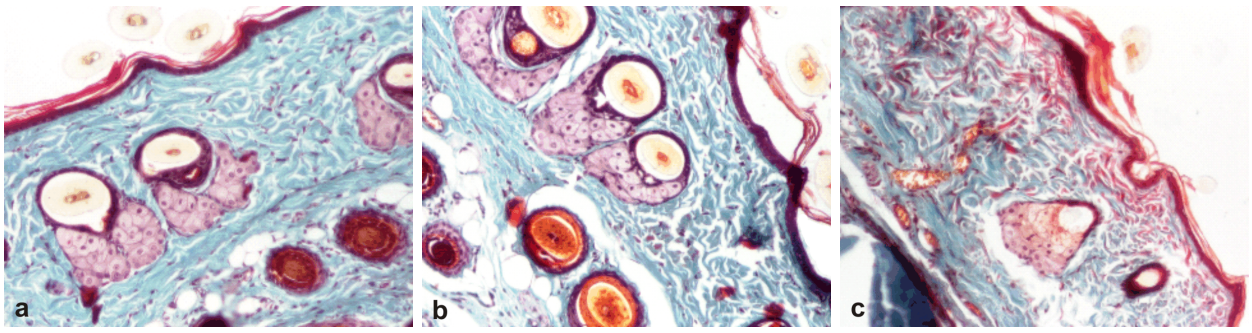
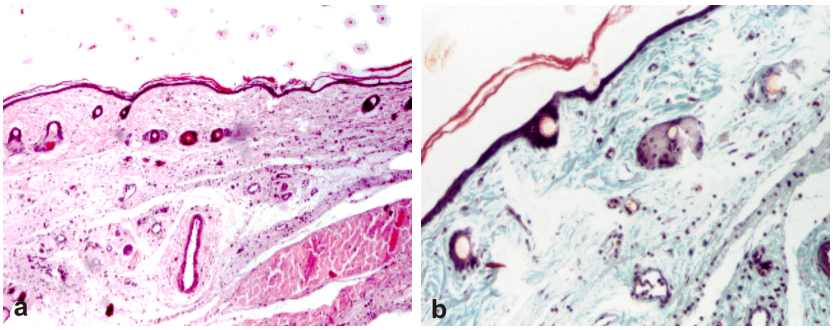


Figure 4 – ***Control. Normal skin, general view: (a) Group C; (b) Group E+C; (c) Group T+C. GS trichrome staining: (a–c) ×100. C: λ-Carrageenan; E: Eutectic mixture of local anesthetics (EMLA); T: Tested substance; GS: Goldner–Szekely.***

Figure 5 – ***Experimental Group C. Skin with mild acute inflammatory reaction, discrete edema in the superficial and deep dermis and mild vascular congestion: (a) Mild inflammation in the dermis; (b) Mild inflammation, edema and vascular congestion in the dermis. HE staining: (a) ×40. GS trichrome staining: (b) ×100. C: λ-Carrageenan; HE: Hematoxylin–Eosin; GS: Goldner–Szekely.***



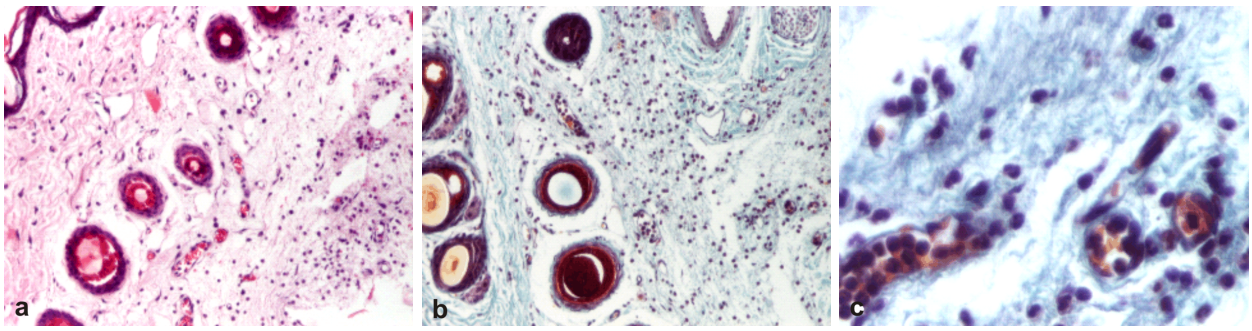


Figure 6 – ***Experimental group E+C. Skin with inflammatory reaction, with diffuse disposition in the superficial and deep dermis (the place of injection), with perivascular disposition, edema and vascular congestion with leukocytosis: (a) Diffuse inflammation in superficial and deep dermis; (b) Diffuse and perivascular inflammation in deep dermis; (c) Vascular congestion with leukocytosis. HE staining: (a) ×100. GS trichrome staining: (b) ×100; (c) ×400. E: Eutectic mixture of local anesthetics (EMLA); C: λ-Carrageenan; HE: Hematoxylin–Eosin; GS: Goldner–Szekely.***

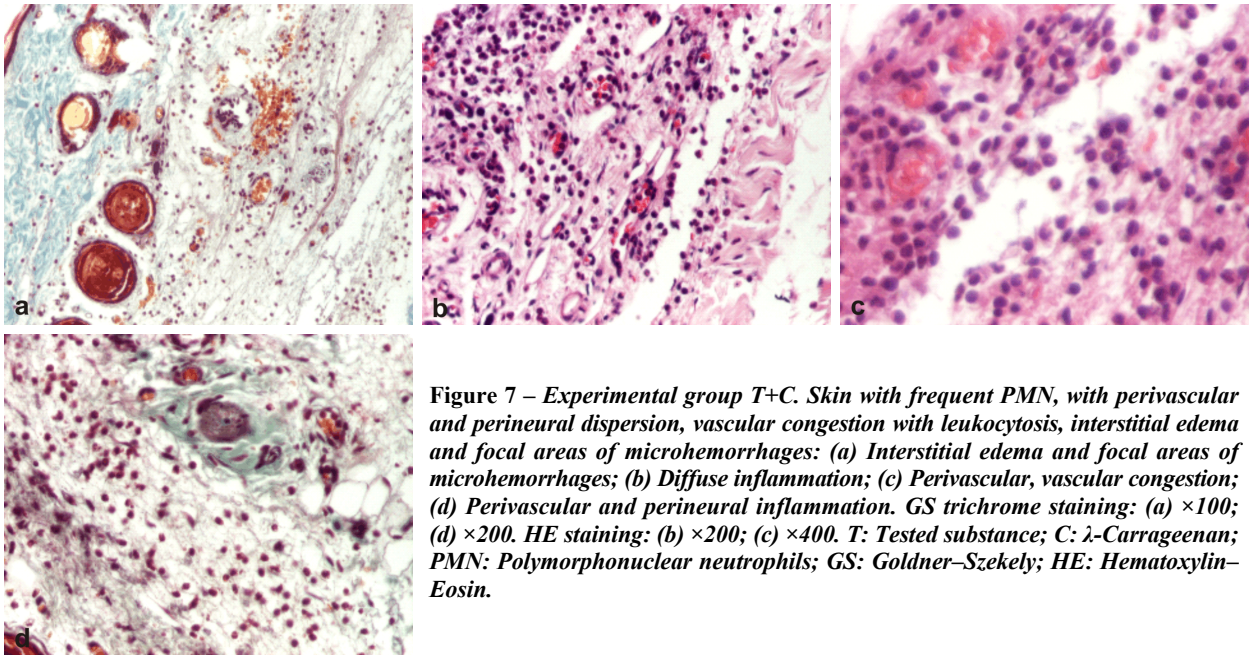


Figure 7 – ***Experimental group T+C. Skin with frequent PMN, with perivascular and perineural dispersion, vascular congestion with leukocytosis, interstitial edema and focal areas of microhemorrhages: (a) Interstitial edema and focal areas of microhemorrhages; (b) Diffuse inflammation; (c) Perivascular, vascular congestion; (d) Perivascular and perineural inflammation. GS trichrome staining: (a) ×100; (d) ×200. HE staining: (b) ×200; (c) ×400. T: Tested substance; C: λ-Carrageenan; PMN: Polymorphonuclear neutrophils; GS: Goldner–Szekely; HE: Hematoxylin–Eosin.***

Discussions

In current investigations, animal models are used to assess the analgesic potential or to determine the inflammatory changes of different drugs. The most used substance that induces inflammatory pain is λ-carrageenan [21]. In our study, we used this model by subcutaneous administration of saline solution of λ-carrageenan in order to induce this type of inflammation.

There are currently known two approaches in order enhance the absorption of topically applied drugs at the skin level: the first one uses diffusion enhancers and the second utilizes a device or vehicle which take advantage of drug delivery into the skin without disturbing the physiochemical properties of superficial layer. A proper enhancer should be non-allergenic or non-irritating, non-toxic, a suitable solvent for the drug and also pharmacologically inactive [22]. Therefore, our study attempts to propose a compound that has the characteristics mentioned above, with effectiveness in pediatric or adult clinical practice.

EMLA is an oil-in-water emulsion mixture based of two well-known local anesthetics Lidocaine and Prilocaine, developed in order to anesthetize intact skin. It is necessary to apply EMLA under occlusive bandages, which helps penetration into the layers of the skin. The suggested application interval for appropriate analgesia is at least one hour, but the time of analgesia is influenced by the density of the blood vessels in the confined area [22, 23]. Our data proved that the effect of the tested substance, for which there is no need of occlusive dressings, was higher at the two-hours testing than that of EML. The peak effect occurs within the range 15–30 minutes, then diminishes, but does not disappear neither at two hours.

The EMLA local effects are transitory blanching followed by erythema, but edema and itching were also observed. Many applications determine intensified local reactions but they disappear after ending the administration [22]. The histopathological exam performed in our study revealed a more intense inflammatory reaction in the group of animals who received λ-carrageenan and EMLA than in the group only with λ-carrageenan, suggesting a

pro-inflammatory local effect of EMLA. The results were similar in the group of animals were the tested substance was associated with λ-carrageenan.

Our data regarding the effectiveness of topical EMLA administration compared to other Lidocaine-containing compounds are consistent with most of the literature data [24–28], regardless the way of administration. Administration of Lidocaine by skin contact is considered easier compared to injection, having the same effect. There is one study in the literature that shows that administration of Lidocaine alone has a lower effect than that of EMLA [29], in contradiction with our data. On the other hand, Cozzi *et al.*, in a study published in 2017, showed that warm Lidocaine administration has superior EMLA effect [30]. Within this context, we believe that further research in the area are still needed to elucidate the exactly morpho-physiological features of topical Lidocaine compounds administration.

Conclusions

Significant research related to skin permeation mechanisms have been done over the last years. Their results are reflected in our days by the developments of new formulations and procedures that enhance drug delivery, essential for local and or systemic effects. In our study, the outcomes obtained with the algesimeter, correlated by those obtained with the cold plate test and the hot plate test proved that the tested substance has superior analgesic properties compared to EMLA. The local histopathological assessment showed no significant differences regarding the morphological features associated to inflammatory reaction between the groups to which EMLA and the tested substance were associated, but further studies are needed in the future.

Conflict of interests

The authors report no conflict of interests.

Acknowledgments

This study was supported by a grant from the Romanian National Authority for Scientific Research (CNCS – UEFISCDI), project number PN-II-PT-PCCA-2013-4-2210 (NANODERMA). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

[1] Anitescu M, Benzon HT, Argoff CE. Advances in topical analgesics. Curr Opin Anaesthesiol, 2013, 26(5):555–561.
[2] Argoff CE. Topical analgesics in the management of acute and chronic pain. Mayo Clin Pro, 2013, 88(2):195–205.
[3] Barkin RL. The pharmacology of topical analgesics. Postgrad Med, 2013, 125(4 Suppl 1):7–18.
[4] Breivik H, Eisenberg E, O'Brien T; OPENMinds. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. BMC Public Health, 2013, 13(1):1229.
[5] Cassuto J, Sinclair R, Bonderovic M. Anti-inflammatory properties of local anesthetics and their present and potential clinical implications. Acta Anaesthesiol Scand, 2006, 50(3):265–282.
[6] Draganescu D, Ibanescu C, Tamba BI, Andritoiu CV, Dodi G, Popa MI. Flaxseed lignan wound healing formulation: characterization and *in vivo* therapeutic evaluation. Int J Biol Macromol, 2015, 72:614–623.
[7] Birol Muhammet E, Kocamanoğlu İS, Bozkurt A, Bilge S, Çetinoğlu EÇ. Evaluation of postoperative anti-nociceptive efficacy of intrathecal dexketoprofen in rats. Balkan Med J, 2016, 33(3):245–251.
[8] Grosse-Steffen T, Krämer M, Tuschy B, Weiss C, Sütterlin M, Berlit S. Topic anaesthesia with a eutectic mixture of Lidocaine/Prilocaine cream after elective Caesarean section: a randomised, placebo-controlled trial. Arch Gynecol Obstet, 2017, 296(4):771–776.
[9] Gunn A, Bobeck EN, Weber C, Morgan MM. The influence of non-nociceptive factors on hot-plate latency in rats. J Pain, 2011, 12(2):222–227.
[10] Hardman MJ, Sisi P, Banbury DN, Byrne C. Patterned acquisition of skin barrier function during development. Development, 1998, 125(8):1541–1552.
[11] Hashmi JA, Baliki MN, Huang L, Parks EL, Chanda ML, Schnitzer T, Apkarian AV. Lidocaine patch (5%) is no more potent than placebo in treating chronic back pain when tested in a randomised double blind placebo controlled brain imaging study. Mol Pain, 2012, 8:29.
[12] Ji Y, Guo L, Zhang Y, Liu Y. Comparison of topical anesthesia and subcutaneous infiltration anesthesia with regard to effect of plasma skin regeneration system. J Cosmet Laser Ther, 2015, 17(6):330–334.
[13] Jyväkorpi M. A comparison of topical EMLA cream with Bonain's solution for anesthesia of the tympanic membrane during tympanocentesis. Eur Arch Otorhinolaryngol, 1996, 253(4–5):234–236.
[14] Mick G, Correa-Illanes G. Topical pain management with the 5% Lidocaine medicated plaster – a review. Curr Med Res Opin, 2012, 28(6):937–951.
[15] Peptu C, Rotaru R, Ignat L, Humelnicu AC, Harabagiu V, Peptu CA, Leon MM, Mitu F, Cojocaru E, Boca A, Tamba BI. Nanotechnology approaches for pain therapy through transdermal drug delivery. Curr Pharm Des, 2015, 21(42):6125–6139.
[16] Sawynok J. Topical analgesics for neuropathic pain: preclinical exploration, clinical validation, future development. Eur J Pain, 2014, 18(4):465–481.
[17] Strain MM, Vineyard MA, Roberto ME, Brumley MR. Effectiveness of topical anesthetics on reducing tactile sensitivity in the paws of newborn rats. Dev Psychobiol, 2014, 56(1):126–132.
[18] Milani AS, Zand V, Abdollahi AA, Froughreyhani M, Zakeri-Milani P, Jafarabadi MA. Effect of topical anesthesia with Lidocaine–Prilocaine (EMLA) cream and local pressure on pain during infiltration injection for maxillary canines: a randomized double-blind clinical trial. J Contemp Dent Pract, 2016, 17(7):592–596.
[19] Suvarna SK, Layton C, Bancroft JD (eds). Bancroft's theory and practice of histological techniques. 8th edition, Elsevier, 2019.
[20] Mondal SK. Manual of histological techniques. 1st edition, Jaypee Brothers Medical Publishers Pvt Ltd., 2017.
[21] Fehrenbacher JC, Vasko MR, Duarte DB. Models of inflammation: carrageenan- or complete Freund's adjuvant (CFA)-induced edema and hypersensitivity in the rat. Curr Protoc Pharmacol, 2012, Chapter 5:Unit5.4.
[22] Tadicherla S, Berman B. Percutaneous dermal drug delivery for local pain control. Ther Clin Risk Manag, 2006, 2(1):99–113.
[23] Biran V, Gourrier E, Cimerman P, Walter-Nicolet E, Mitanchez D, Carbajal R. Analgesic effects of EMLA cream and oral sucrose during venipuncture in preterm infants. Pediatrics, 2011, 128(1):e63–e70.
[24] Ata N, Bülbül T, Demirkan A. Comparison of EMLA cream and Lidocaine injection for local anaesthetic before radiofrequency reduction of the inferior turbinates. Br J Oral Maxillofac Surg, 2017, 55(9):917–920.
[25] Sun L, Zhao PP, Guan GT. [Analgesic efficacy of compound Lidocaine cream in the lumbar puncture of children with hematologic tumor]. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 2019, 27(3):991–994.
[26] Abbas AM, Abdel Hafiz HA, Abdelhafez AM, Michael A, Ismail AM. Topical Lidocaine–Prilocaine cream *versus* Lidocaine infiltration for pain relief during repair of perineal tears after vaginal delivery: randomized clinical trial. J Matern Fetal Neonatal Med, 2019, 32(13):2182–2187.

[27] Latsios G, Toutouzas K, Antonopoulos AS, Melidi E, Synetos A, Vogiatzi G, Stasinopoulou A, Tsioufis K, Tsiamis E, Tousoulis D. Anesthetic ointment only (Lidocaine/Prilocaine) instead of injectable local Lidocaine in trans-radial catheterization: a viable no-needle alternative. J Interv Cardiol, 2017, 30(4):382–386.
[28] Kargar R, Aghazadeh-Nainie A, Khoddami-Vishteh HR. Comparison of the effects of Lidocaine Prilocaine cream (EMLA) and Lidocaine injection on reduction of perineal pain during perineum repair in normal vaginal delivery. J Family Reprod Health, 2016, 10(1):21–26.
[29] Horikiri M, Ueda K, Sakaba T. Comparison of EMLA cream and topical Lidocaine tape for pain relief of V-beam laser treatment. J Plast Surg Hand Surg, 2018, 52(2):94–96.
[30] Cozzi G, Borrometi F, Benini F, Neri E, Rusalen F, Celentano L, Zanon D, Schreiber S, Ronfani L, Barbi E. First-time success with needle procedures was higher with a warm Lidocaine and Tetracaine patch than an eutectic mixture of Lidocaine and Prilocaine cream. Acta Paediatr, 2017, 106(5):773–778.

Corresponding authors
Laura Mihaela Trandafir, Lecturer, MD, PhD, Department of Mother and Child – Pediatrics, "Grigore T. Popa" University of Medicine and Pharmacy, 16 University Street, 700115 Iaşi, Romania; Phone +40721–471 041, e-mail: trandafirlaura@yahoo.com

Andrei Luca, Assistant Professor, MD, PhD, CEMEX Laboratory, "Grigore T. Popa" University of Medicine and Pharmacy, 9–13 Mihail Kogălniceanu Street, 700454 Iaşi, Romania; Phone +40757–762 607, e-mail: lc_andrei@yahoo.com

Received: August 14, 2019

Accepted: December 5, 2019