

Viability and intracellular nitric oxide generation in the umbilical cord blood CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations exposed to local anaesthetics

KAROLINA I. KULIŃSKA¹, HANNA BILLERT¹, KRZYSZTOF SAWIŃSKI², KATARZYNA CZERNIAK¹, MACIEJ KURPISZ³

¹Department of Experimental Anaesthesiology, Chair of Anaesthesiology and Intensive Therapy, Poznan University of Medical Sciences, Poznan, Poland

²Department of Haematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poznan, Poland

³Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland

Abstract

Local anesthetics (LAs) are capable of influencing cell viability in systemic immunity and may also modify metabolism of those present in umbilical cord blood (UCB) following obstetric neuraxial analgesia and anaesthesia. Data regarding UCB immature cells, important for the neonate and critical for putative UCB transplantations, are lacking. LAs are capable of stimulating intracellular nitric oxide (NO) in human neutrophils; no information is available concerning newly perpetuated cells and its potential association with viability. The study aimed at assessing the LAs influence on the cell viability and intracellular NO production by UCB CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations. Mononuclear cells separated from UCB samples (n = 19) were incubated with bupivacaine (0.0005, 0.005, 1 mM), lidocaine (0.002, 0.02, 4 mM), and ropivacaine (0.0007, 0.007, 1.4 mM) for 4 h. Flow cytometry was applied for the assessment of cell viability and intracellular NO generation in CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations using annexinV/7-AAD and DAF-2DA stainings, respectively. CD34⁺CD133⁺ cells showed less pronounced late apoptosis and necrosis as compared to CD34⁺CD133⁻ population. Intracellular NO generation was comparable between both cell populations studied. LAs neither influenced cell viability nor changed NO production in either population. LAs do not interfere with viability and intracellular NO generation in the UCB CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations.

Key words: umbilical cord blood, CD34, CD133, cell viability, nitric oxide, local anaesthetics.

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Introduction

In the foetal circulation, mature immune cells are accompanied by relatively abundant immature cell subsets. In case of neonatal pathology, these cells support host resistance and tissue repair additionally providing a prognostic tool [1]. On the other hand, umbilical cord blood (UCB) containing low differentiated, pro-regenerative cells can be applied for allogeneic transplantation and tissue repair in various pathologies, e.g. haematological and autoimmune diseases. Advantages of their applications involve better HLA mismatch toleration and lower risk of graft-versus-host disease as compared to adult cells [2]. UCB is primarily considered a source of hematopoietic stem cells (HSC), which reside in heterogenic CD34⁺ cell fraction. The CD34

antigen, cell surface sialomucin is uniquely expressed on the hematopoietic and progenitor stem cells (HSPC) but also on non-hematopoietic cells and is an accepted marker of stemness and immaturity. It plays a role in cell adhesion, rolling, migration, proliferation, differentiation and apoptosis [3, 4]. The numbers of UCB CD34⁺ cells are critically important for transplantation success of HSCs providing proper conditions to proliferation, mobility, bone marrow homing and engraftment [5, 6]. While subjected to specific expansion techniques, UCB CD34⁺ cell population can provide large amounts of functional innate immune cells, i.e. neutrophils [7]. Regarding the CD34⁺ cells, concomitant assessment of the expression of another immaturity marker in humans, the CD133 molecule (prominin-1), allows to distinguish

Correspondence: Maciej Kurpisz, Institute of Human Genetics Polish Academy of Sciences, 32 Strzeszyńska St., 60-479 Poznan, e-mail: kurpimac@man.poznan.pl
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two functionally distinct cell subpopulations important for further applications: CD34⁺CD133⁻ and CD34⁺CD133⁺. CD133⁺ cells, specifically upregulated in hypoxia and mitochondrial dysfunction, are of significance for immature and tumour cells, cell regeneration and metabolism [8]. Within CD34⁺ cells population, exhibiting CD133, granulocyte-macrophage progenitors (GMP) were exclusively found [6]. Therefore, quality of this specific cell population reflects the HSPC activity of UCB. It has been demonstrated that obstetric and maternal factors could determine CD34⁺ and CD34⁺CD133⁺ cell counts [6, 9]. Potential effects of obstetric anaesthesia have not been explored.

Broad use of regional methods of anaesthesia and analgesia in obstetrics evoked interest in foetal and neonatal implications of these procedures [10]. Agents applied in these techniques, local anaesthetics (LAs), temporally block neuronal ion channels and nociceptive stimulation. However, they may also exert cytotoxic and pro-apoptotic effects in various cell populations. Depending on the clinical or experimental conditions, these actions were proved to be beneficial (e.g., tumour) or detrimental (e.g., neural injury) [11, 12]. LAs instilled locally or neuraxially penetrate the vessels and in pregnant women via placenta reach foetal circulation influencing viability and function of systemic immune cells. We have shown that LAs are capable of inhibiting reactive oxygen species (ROS) generation in neonatal neutrophils even at low concentrations present in foetal blood during mothers' epidural blockade. However, the effect is less pronounced and lasts shorter than in adult cells [10].

In immature cells, it was demonstrated that nitric oxide (NO), a pleiotropic signaling and effector molecule, is involved in cell survival and renewal in a concentration dependent manner [13]. LAs are able to influence NO signaling in immune cells, decreasing or increasing NO endogenous generation and expression of NO synthase (NOS) isoforms depending on the cell type [14, 15]. Whether this process occurs in not finally differentiated cells and correlates with the cell life span remains unclear.

This study aimed at assessing the LAs influence on the viability and intracellular NO production of UCB CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations.

Material and methods

The study was performed according to the Bioethical standards of Helsinki Declaration and its latest revision. After obtaining local Bioethical Commission approval (780/13, 659/15, Poznan University of Medical Sciences) and mothers' written informed consent, the cord blood samples (18 ml) from healthy, full term neonates ($n = 19$) were drawn immediately after cord clamping and proceeded within 1 h. Mothers were non-smoking, healthy individuals neither receiving any medication nor requesting neuraxial blockade for labour pain control.

Mononuclear cell isolation

Mononuclear cells (MNC) were isolated from UCB by density gradient centrifugation using GradiSol L ($d = 1.077 \text{ g/cm}^3$; Aqua-Med, Poland) and centrifuged at 400 g, RT, for 30 min. The buffy coat was collected, PBS-washed, centrifuged and resuspended in 2 ml Hank's balanced salt solution without $\text{Ca}^{2+}/\text{Mg}^{2+}$ (HBSS; Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland), and then counted using Bürker chamber. MNC purity and viability on Trypan blue dye exclusion were $> 95\%$ and $> 98\%$, respectively. Samples of 3×10^6 cells were used for further analysis. No erythrocyte lysis was necessary.

Incubation with local anaesthetics

Stock solutions of 5 mM bupivacaine (Bupivacaine hydrochloride monohydrate; Sigma-Aldrich, MO, USA), 16 mM lidocaine (Lidocaine hydrochloride monohydrate; Sigma-Aldrich), and 7 mM ropivacaine (Ropivacaine hydrochloride monohydrate; Sigma-Aldrich) prepared from water dissolved agents were sterilized and refrigerated until use. Before experiments, LAs were diluted in HBSS to obtain final equipotent concentrations: 0.0005, 0.005 and 1 mM bupivacaine; 0.002, 0.02, and 4 mM lidocaine; and 0.0007, 0.007 and 1.4 mM ropivacaine. The lowest concentrations of bupivacaine and ropivacaine reflected those observed in foetal circulation during epidural labour analgesia [16]. MNC were incubated with LAs under standard conditions (37°C ; $5\% \text{ CO}_2$; HeraCell 150; ThermoScientific, MA, USA) for 4 h.

Flow cytometry

After incubation with LAs, MNC were centrifuged and incubated with monoclonal antibodies conjugated with fluorochromes: anti-CD34- PE (clone AC136, Miltenyi Biotec, Bergisch Gladbach, Germany) and anti-CD133- APC (clone 293C3, Miltenyi Biotec, Bergisch Gladbach, Germany) in the dark, 4°C , for 10 min. Cells were washed twice with cold PBS supplemented with 2 mM EDTA and 0.5% FCS. Progenitor cells were identified by flow cytometry (FACS CantoII; BD Biosciences, San Jose, CA, USA) based on forward (FSC) vs. side scatter (SSC) characteristics and the presence of surface antigens.

Cell viability

Apoptotic cells were assessed using annexinV-FITC/7-AAD assay (BD Pharmingen, USA). After antibody staining, cells were washed twice with cold PBS, resuspended in Binding Buffer, incubated with Annexin V-FITC ($5 \mu\text{l}$) and 7-AAD ($5 \mu\text{l}$) in the dark, RT for 15 min, and then analysed by flow cytometry within 1 h.

In both studied cell populations, viable (Annexin V-/7AAD-), early apoptotic (Annexin V+/7AAD-), late apo-

ptotic (Annexin V+/7AAD+), and necrotic cells (Annexin V-/7-AAD+) were distinguished. Compensation was set up and calculated using FACS Diva Software (BD Biosciences).

Intracellular NO production

Mononuclear cells were incubated with 2.5 μ M DAF-2DA (4,5-Diaminofluorescein diacetate solution; Sigma-Aldrich) in dark, RT, for 2 h. Fluorescence from triazolo-fluorescein (DAF-2T) reflected intracellular NO generation. DAF-2T positive events were gated as the CD34+CD133- and CD34+CD133+ cell populations with high fluorescence in FL1/DAF-2T FITC channel. Gates of high fluorescence were set in comparison to non-fluorescent control.

Samples of 2×10^6 MNC were collected. The number of analysed cells was the total number of cells per 2×10^6 MNC.

Statistics

Statistical analysis was performed using Statistica version 12 (StatSoft, Tulsa, OK, USA), JumpPro (jmp-11.0) 32 bit 11.0.0. (SAS Institute), and GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, USA).

Multifactorial analysis of variance (ANOVA) was applied for the assessment of LAs impact on cell viability and NO production. Percentage data of cell viability were arcsine transformed to obtain normal distribution. Effects of independent factors: 1) 'cell population' – CD34+CD133- and CD34+CD133+ cells, 2) 'anaesthetic' – bupivacaine, lidocaine, ropivacaine, and 3) 'concentration-category' – concentrations of LAs categorized by their potency: the lowest, middle, the highest, were evaluated. Additionally, interactions between the factors were assessed.

Results

Obstetric and newborn data are presented in Table 1.

Cytometric cell phenotype identification and viability assessment are shown in Figure 1A-F.

Viability of UCB CD34+CD133- and CD34+CD133+ cells

UCB CD34+CD133- cell population showed higher percentage of late apoptotic and necrotic cells as compared to CD34+CD133+ cells ($p = 0.0012$ and 0.0064 , respectively, multifactorial ANOVA; Fig. 1F, Table 2). Higher percentage of viable CD34+CD133+ cells failed to reach statistical significance ($p = 0.0652$).

Nitric oxide generation in UCB CD34+CD133- and CD34+CD133+ cells

Nitric oxide production revealed in CD34+CD133- and CD34+CD133+ UCB cell populations was similar ($p > 0.05$; Fig. 1G).

Effects of local anaesthetics on cell viability and nitric oxide generation

Local anesthetics did not affect the viability of UCB cells of both phenotypes studied. The effect was comparable between bupivacaine, lidocaine, and ropivacaine. The agents did not change the percentage of viable, early apoptotic, late apoptotic and necrotic cells (Fig. 2A).

Viability of UCB CD34+CD133- and CD34+CD133+ cells exposed to particular LAs studied were comparatively presented in Table 2 reflecting significant interaction between the factors 'population' and 'viability'. All applied concentrations of particular LA were collectively analysed. Regarding UCB CD34+CD133+ cell population, percentage of live cells was higher than that of CD34+CD133- cells, and percentages of necrotic and apoptotic (early and late) cells were consequently lower under the exposure to each anaesthetic tested.

Table 1. Obstetric characteristics and the newborn data

Maternal and obstetric data	
Primigravida (n)	5
Gestational age (weeks)	39 (38-40)
Time (min)	
Stage I labour	271 \pm 120
Stage II labour	15 (12-35)
Maternal body temperature during II stage of labour ($^{\circ}$ C)	36.5 (36.3-36.5)
Neonatal data	
Male (n)	9
Body mass (g)	3321 \pm 298
Placenta weight (g)	578 \pm 117
Apgar score 1 st min	10 (10-10)
Apgar score 5 th min	10 (10-10)
Cord blood gas and pH post labour	
Umbilical artery	
pH _a	7.27 \pm 0.08
P _a O ₂ (mmHg)	21.6 \pm 8.1
P _a CO ₂ (mmHg)	51.7 \pm 11.4
BE _a	-4.8 \pm 3.3
Umbilical vein	
pH _v	7.34 \pm 0.07
P _v O ₂ (mmHg)	23.2 \pm 7.8
P _v CO ₂ (mmHg)	43.5 (33.6-49.8)
BE _v	-4.0 (-5.5 to -2.7)

Categorized data are presented as numbers, data fitting normal distribution are presented as means \pm standard deviation; non-normally distributed data are given as median values (interquartile range). BE – base excess

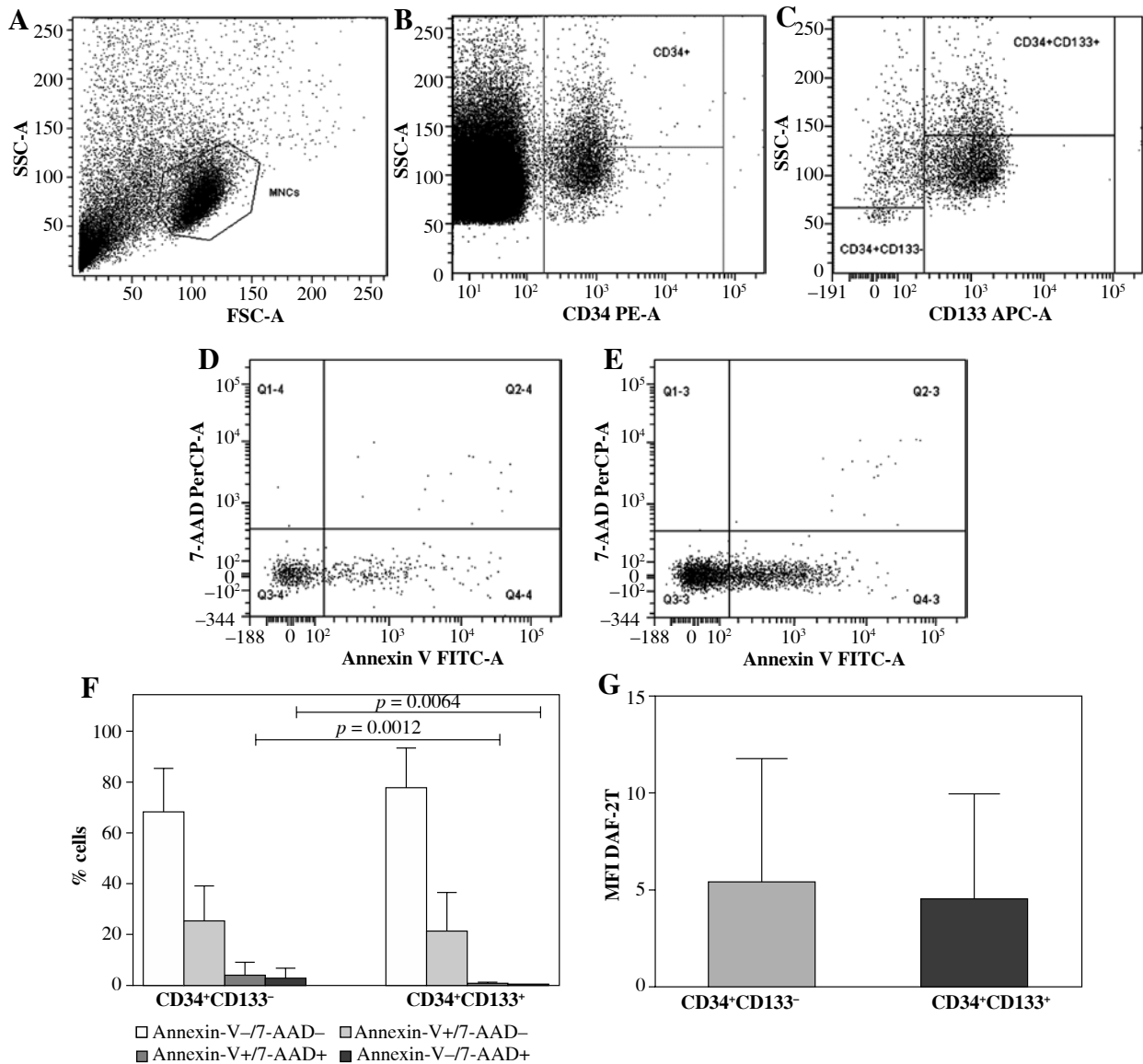


Fig. 1. Cytometric identification, viability, and intracellular nitric oxide (NO) generation in umbilical cord blood (UCB) CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations (*n* = 19). **A**) Representative dot plot of UCB mononuclear cells (MNCs) identified by forward and side scatter characteristics (FSC and SSC, respectively). 2 × 10⁶ MNC samples were evaluated. **B**) Progenitor cells were separated from MNCs using CD34 gate. **C**) CD133⁺ and CD133⁻ subsets were identified. Viable (Annexin V⁻/7-AAD⁻), early apoptotic (Annexin V⁺/7-AAD⁻), late apoptotic (Annexin V⁺/7-AAD⁺) and necrotic (Annexin V⁻/7-AAD⁺) cells were distinguished in **(D)** CD34⁺CD133⁻ and **(E)** CD34⁺CD133⁺ cell populations. **F**) Percentages of viable (white bars), early apoptotic (light grey bars), late apoptotic (dark grey bars) and necrotic (black bars) CD34⁺CD133⁻ and CD34⁺CD133⁺ cells. More details of multifactorial ANOVA analysis have been summarized in Table 2. **G**) Intracellular NO production in CD34⁺CD133⁻ and CD34⁺CD133⁺ cells was assessed by flow cytometry and is expressed as mean fluorescence intensity (MFI) of triazolofluorescein (DAF-2T). **F, G**) Data are the mean ±SD

Intracellular NO production was unaffected under the exposure to all LAs tested in both cell populations studied. No differences between UCB CD34⁺CD133⁻ and CD34⁺CD133⁺ cells in this aspect have been found (Fig. 2B).

Discussion

In the present study, we investigated effects of commonly used aminoamide-type LAs, bupivacaine, lidocaine and ropivacaine, on the cell viability and intracellular NO generation among immature UCB cell populations

Table 2. Percentage of viable (Annexin V-/7-AAD-), early apoptotic (Annexin V+/7-AAD-), late apoptotic (Annexin V+/7-AAD+), and necrotic (Annexin V-/7-AAD+) UCB CD34+CD133- and CD34+CD133+ cells exposed to bupivacaine, lidocaine and ropivacaine

	Control				Bupivacaine				Lidocaine				Ropivacaine			
	CD34+CD133-	CD34+CD133+	F _{1,34}	p-value	CD34+CD133-	CD34+CD133+	F _{1,140}	p-value	CD34+CD133-	CD34+CD133+	F _{1,36}	p-value	CD34+CD133-	CD34+CD133+	F _{1,140}	p-value
Annexin V-/7-AAD-	78 ±16	68 ±17	3.63	0.0652	77 ±15	67 ±16	14.97	0.0002	77 ±14	68 ±14	16.30	<0.0001	77 ±16	68 ±15	12.43	0.0006
Annexin V+/7-AAD-	21 ±15	25 ±14	0.85	0.3618	22 ±15	27 ±14	4.75	0.0310	23 ±15	27 ±14	4.24	0.0415	23 ±15	27 ±13	4.10	0.0447
Annexin V+/7-AAD+	0.6 ±0.5	4 ±5	12.47	0.0012	0.5 ±0.5	4 ±6	63.48	<0.0001	0.6 ±0.7	4 ±5	67.02	0.0001	0.7 ±0.8	4 ±5	60.96	<0.0001
Annexin V-/7-AAD+	0.2 ±0.3	2.5 ±4.3	8.45	0.0064	0.2 ±0.2	1 ±2	35.74	<0.0001	0.2 ±0.2	2 ±3	43.47	<0.0001	0.2 ±0.2	1 ±2	43.22	<0.0001

Data are the mean ±SD. Multifactorial ANOVA, interaction of the factors 'population' and 'viability'; for particular LAs, all concentrations applied were collectively analysed. F – F ratio

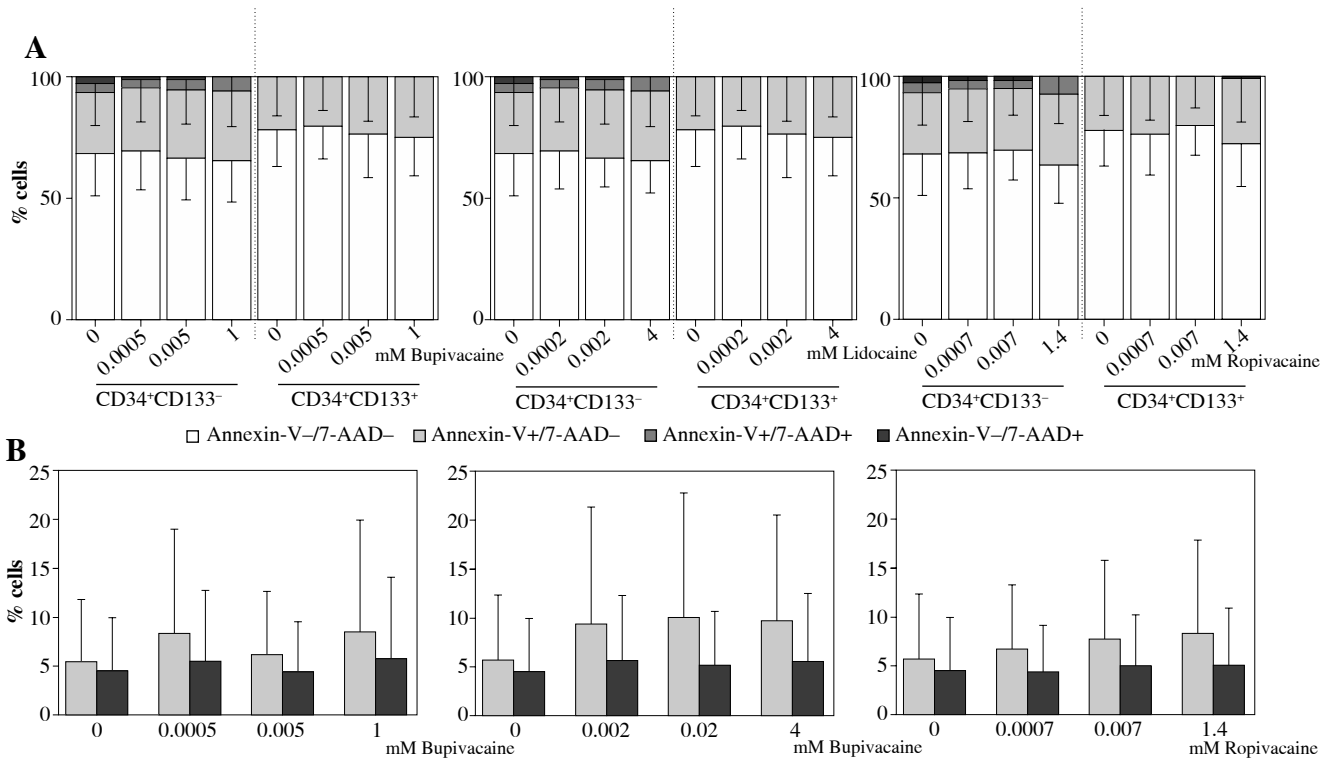


Fig. 2. Effects of local anaesthetics (LAs) on viability and intracellular nitric oxide (NO) generation in UCB CD34+CD133- and CD34+CD133+ cell populations (n = 19). **A**) Percentages of viable (Annexin V-/7-AAD-; white bars), early apoptotic (Annexin V+/7-AAD-, light gray bars), late apoptotic (Annexin V+/7-AAD+; dark gray bars) and necrotic (Annexin V-/7-AAD+; black bars) cells evaluated in CD34+CD133- and CD34+CD133+ populations incubated with bupivacaine, lidocaine and ropivacaine for 4 h. Graphical representation of significant interaction determined by multifactorial ANOVA between the factors of 'population' and 'viability'; all applied concentrations of particular LA were collectively assessed. In CD34+CD133+ cell subset, percentage of live cells was higher than that of CD34+CD133- cells, and percentages of necrotic and apoptotic cells were consequently lower under the exposure of each LA tested. Data are the mean ±SD. Further details are shown in Table 2. **B**) Intracellular NO production in CD34+CD133- and CD34+CD133+ cells exposed to LAs was assessed by flow cytometry and is expressed as mean fluorescence intensity (MFI) of triazolo-fluorescein (DAF-2T). Data are the mean ±SD, p > 0.05

CD34⁺CD133⁻ and CD34⁺CD133⁺. We observed significant differences in late apoptosis and necrosis between these cell fractions, however, we failed to detect any significant influence of the agents on the phenomena studied.

Viability of CB CD34⁺ cells varied substantially (over 80% vs. 40%), depending on blood samples delivery and mode of cell isolation. Results obtained in this study corresponded with previously reported values [17]. We observed that in the CD34⁺CD133⁺ cells, late apoptosis and necrosis were significantly less frequent than in CD34⁺CD133⁻ cell population, indicating higher survival potential of the former population. Percentages of early apoptosis corresponding with functional deficits, like decreased clonogenic and transmigration capacity were, however, comparable [18]. Our results indicate that CD34⁺CD133⁺ phenotype present with less pronounced propensity to cell death and are consistent with the resistance to apoptosis of CD133⁺ phenotype [8, 19]. In human UCB MNC subset, CD133⁺ phenotype showed better radioresistance compared with CD133⁻ cells with the low radiation induced apoptosis and distinct repair signalling [20]. Relative insensitivity to apoptosis of UCB progenitors was reported to depend on active upregulation of caspase 8 and NF-κB-related pathway [19], which in our study analysing UCB cells exposed to inflammatory conditions of natural labour might have been of importance.

In the present study, no difference in intracellular NO generation between both cell populations studied could be observed. NO generation is important for CD34⁺ cell biology. In adult peripheral blood CD34⁺ fraction of MNC, NO was produced twice as much as in CD34⁻ cells [21]. Association between NO generation and cell death is also a complex issue. Pro- or anti-apoptotic actions of NO in not finally differentiated cells were demonstrated to depend on the mediator amount and/or its source, endogenous or extracellular [13]. In bone marrow CD34⁺ cells, Fas-mediated apoptosis was connected with the activity of inducible NOS2 isoform providing higher amounts of NO than the other two constitutive NOS isoforms [22]. In our study, specific inflammatory and hormonal milieu of natural labour and exposure of the studied cell populations to a number of stimuli including cytokines and hormones present in UCB could also influence NO intracellular generation, i.e., upregulating the NOS2 [23]. Human CD34⁺ stem cells exposed to extracellular NO produced by M1-macrophages which demonstrated prevalent NOS2 activity, presented increased apoptosis and impaired self-renewal [24]. On the other hand, in UCB hematopoietic cells, extracellular NO may also inhibit apoptosis and upregulate a number of transcription factors connected with CD34 [25, 26]. NO is also able to activate cell signaling pathways important for *ex vivo* expansion of CB HSC [27, 28]. In view of the complexity of NO influence on UCB CD34⁺ cell survival, a deeper insight identifying expression and activity of specific NOS isoforms should be warranted.

In this study, no significant influence of LAs, bupivacaine, lidocaine and ropivacaine on UCB CD34⁺CD133⁻ and CD34⁺CD133⁺ cell viability was detected. These findings, especially important from a clinical point of view, suggest that in perinatal settings neuraxial blockades should not negatively influence UCB cells important for the neonate and/or for banking purposes.

LAs were repeatedly shown to be able to induce cell death in various cell populations. In immune cells, these agents may exert either cytotoxic or stimulatory effects [29]. As shown in Jurkat cells, association between LAs cytotoxicity and agents' characteristics, i.e., lipophilicity and clinical potency, was not so evident as in neuronal cells. Structural features like ester or amide linkage and stereospecificity were also of no significance [30]. In clinical conditions, transient apoptotic effect of epidural anaesthesia using lidocaine and ropivacaine in peripheral blood monocytes and lymphocytes was observed [31]. In immature cells, like preadipocytes and mesenchymal stem cells (MSC), cytotoxicity was well documented [32-35]. Commonly used aminoamide-type LAs decreased viability of MSC in agent-, concentration- and time-dependent manner. In this study, underlying reasons for lack of LAs effects on the studied UCB CD34⁺ cell subpopulations viability might involve: 1) specific experimental conditions applied, agents' concentrations and time of exposure which were subjected to clinical conditions, 2) distinct UCB CD34⁺ cell biology, and 3) cell exposure to specific inflammatory and hormonal milieu of natural labour. In murine MSC, ropivacaine at 100 and 250 μM activated early apoptosis, with no detectable effect on late apoptosis only after 24-h exposure [33]. In human MSC, apoptosis was observed as early as after 40 min incubation, however, agents were applied at high concentrations of molar range [34]. Delayed effects after 96 h since 1 h exposure were reported also for molar concentrations [35]. Concentrations applied in this study were much lower, the lowest concentrations reflected real amounts of agent in UCB following maternal epidural blockade, up to the highest ones, millimolar assumed as reference values. Exposure time of 4 also referred to clinical conditions [36]. Reported mechanisms responsible for detrimental effects on MSC viability differed between the agents applied and involved mitochondrial dysfunction typical for lidocaine (also demonstrated in human neutrophils) and depletion of intracellular calcium stores observed under bupivacaine exposure [33, 34, 37]. On the other hand, LAs are able to increase or inhibit ROS generation and to increase intracellular pH (pHi) by blocking voltage-gated proton (VDPC) channels, whereas in CD34⁺ cells it was demonstrated that early apoptosis is associated with ROS stimulation and pHi decrease [38, 39]. Extracellular acidosis, typical for UCB milieu and also documented in this study, had no effect on the viability of human MSC in the presence of LAs [35]. It is worth mentioning that cytotoxic effects of LAs in human MSC was

demonstrated only in monolayer cultures and not in the whole tissue samples, indicating that microenvironment of the experiment may be critical for the results obtained [35]. As CD34⁺ phenotype prevents adhesion, this feature might possibly contribute to a lack of cytotoxic effects observed in this study [3, 35]. Eventually, natural labour and delivery is connected with delayed neutrophil apoptosis in maternal blood and in UCB cells [40]; this aspect of apoptosis resistance in UCB immature cells should also be considered. Despite no effect on LAs on cell viability, using multifactorial ANOVA, we could observe consistent differences between two studied cell populations concerning viability parameters. This observation supports earlier described distinct reactivity of CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations with propensity to better survival of CD133⁺ phenotype.

Originally, we suspected that NO signalling could possibly interact with the viability of UCB CD34⁺ cells exposed to LAs. In contrast to our previous findings showing upregulation of intracellular NO generation in UCB neutrophils by LAs, we could not observe here any alteration in NO production under the influence of all LAs tested. This preliminary observation warrants further analysis elucidating specific cellular sources of NO generation.

We are aware of some limitations. Firstly, we applied limited phenotyping of the studied cell populations, not allowing to discriminate between different progenitor subsets. However, for the purpose of this study more detailed assessment was not essential, as we wanted to answer a general question of the influence of LAs on the viability of CD34⁺ cells. In the literature, exploration of different obstetric factors on the HSPC content in CB was also addressed [6]. Secondly, we applied limited concentration of LAs in order to address typical clinical situation. Thirdly, the study was performed using UCB from natural deliveries exposed to labour-induced systemic inflammatory reaction, and in many reports UCB collection occurred during planned caesarean section, in which a response to LAs could be different. Fourthly, our experiments were performed under normoxic conditions, while physiological hypoxia and acidosis *in situ* might have influenced cell reactivity.

Our study demonstrates no effects of LAs on the viability of UCB CD34⁺CD133⁻ and CD34⁺CD133⁺ cell populations which may determine their transplantogenic value. The results obtained are important for both basic biology of these cells and also may have clinical implications. Our observations indicate that regional anaesthetic techniques for labour pain control and/or surgical delivery do not have negative impact on UCB cell survival and as such should not be of concern.

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Authors inform that current affiliation of Karolina I. Kulińska is Department of Experimental Anaesthesiology, Chair of Anaesthesiology and Intensive Therapy, Poznan University of Medical Sciences, Poznan, Poland; Hanna Bilert – Clinics of Anaesthesiology, Intensive Therapy and Pain Management, Chair of Anaesthesiology and Intensive Therapy, Poznan University of Medical Sciences, Poznan, Poland; Katarzyna Czerniak – Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland.

The authors declare no conflict of interest.

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