The influence of bupivacaine on the whole blood chemiluminescence in laboring women – a preliminary report

Wpływ bupiwakainy na chemiluminescencję krwi pełnej kobiet rodzących – doniesienie wstępne

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Abstract

Objective: *Local anesthetics are able to inhibit inflammatory phenomena. The influence of bupivacaine broadly applied in obstetrical anesthesia on the reactive oxygen species (ROS) production is a matter of controversy. The study was aimed at elucidation of the influence of racemic bupivacaine on the opsonized zymosan (OZ) stimulated-peripheral blood chemiluminescence (CL) in laboring women, reflecting the reactive oxygen species (ROS) production associated with phagocytosis.*

Material and methods: *Blood samples drawn from 8 healthy parturients in active spontaneous labor and from 5 healthy non-pregnant controls were incubated with the 0.3, 30, and 3000 μM bupivacaine concentrations and then luminol-dependent OZ-stimulated whole blood CL was assessed.*

Results: *Bupivacaine depressed CL; however, the inhibitory effect was significant only at the highest, clinically irrelevant concentration (3000μM), in parturients being comparable to that observed in non-pregnant women.*

Conclusions: *Bupivacaine at clinically relevant concentrations does not influence ROS production accompanying phagocytosis in peripheral blood of laboring women. The effect is comparable in parturients and non-pregnant controls.*

Key words: **labor** / **bupivacaine** / **chemiluminescence** /

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Streszczenie

Cel pracy: *Anestetyki lokalne mają zdolność hamowania procesów zapalenia. Wpływ bupiwakainy, anestetyku szeroko stosowanego w anestezjologii położniczej, na produkcję reaktywnych form tlenu (RFT) jest przedmiotem kontrowersji. Celem pracy była ocena wpływu racemicznej bupiwakainy na chemiluminescencję (CL) krwi obwodowej kobiet rodzących stymulowaną opsonizowanym zymosanem (OZ), odzwierciedlającą produkcję reaktywnych form tlenu (RFT) towarzyszącą procesowi fagocytozy.*

Materiał i metody: *Krew pełna pobrana od zdrowych 8 rodzących i 5 kobiet niecięŻarnych była inkubowana z bupiwakainą w stężeniach 0,3; 30; i 3000 μM, a następnie oceniano zależną od luminolu chemiluminescencję (CL) krwi pełnej stymulowanej OZ.*

Wyniki: *Bupiwakaina hamowała CL, jednak istotny efekt obserwowano dopiero przy zastosowaniu najwyższego badanego stężenia (3000μM). Efekt ten był porównywalny u rodzących i kobiet niecięŻarnych.*

Wniosek: *Bupiwakaina w stężeniach istotnych klinicznie nie wpływa na produkcję RFT towarzyszącą procesowi fagocytozy przez komórki krwi obwodowej kobiet rodzących. Efekt ten jest porównywalny u rodzących i kobiet niecięŻarnych.*

Słowa kluczowe: **poród** / **bupiwakaina** / **chemiluminescencja** /

Introduction

Parturition is physiologically characterized by upregulated both local and systemic inflammatory processes, involving a redox balance shift towards increased oxidative stress-related phenomena and leukocyte priming [1, 2, 3]. Peripheral blood neutrophils of laboring women show upregulation of some surface receptors and delayed apoptosis [1]. Consequently, these cells might possibly differently react to various pharmacological signals and drugs, like local anesthetics (LA).

Local anesthetics (LA) which penetrate to systemic circulation during neuraxial analgesia or anesthesia are able to exert anti-inflammatory and immunosuppressive effects by mechanisms involving ion channel modulation (but not Na^+ influx) and inhibition of Gq proteins of inflammatory cells, i.e. phagocytes [4]. Among other functions, the compounds are also able to inhibit phagocytosis and concomitant respiratory burst with the increased production of reactive oxygen species (ROS), serving the oxygen-dependent pathogen degradation and constituting main functional characteristics of phagocytic cells like neutrophils and monocytes crucial for host defense [5].

However, regarding bupivacaine, a long acting amide local anesthetic frequently used in obstetrical analgesia and anesthesia, available data on the inhibition of ROS production and phagocytosis are conflicting [6-14]. Whether LA administered to laboring women would significantly modulate the inflammatory cell properties, i.e. phagocytosis and respiratory burst, which would influence susceptibility to infection, is actually not known.

Objective

The study was aimed at elucidation of a possible influence of racemic bupivacaine on ROS production connected with phagocytosis by peripheral blood phagocytes as measured by opsonized zymosan (OZ) stimulated whole blood chemiluminescence (CL) in laboring women.

Material and methods

Reagents and apparatus

Bupivacaine hydrochloride, luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione), and zymosan A were purchased from Sigma, calf serum and phosphate buffered saline (PBS) were obtained from Biomed, Lublin.

Bupivacaine was dissolved in PBS ex tempore. Zymosan particles were opsonized according to Labedzka et al. [15], stock solution 10 mg mL^{-1}. Luminol was dissolved in borate buffer (pH 10), end concentration was 1 mM.

Chemiluminescence was measured with use of luminometer LKB 1250 (Bioorbit, Denmark).

Samples were kept at 37°C in thermoblock TB 951U (JW Electronic, Poland).

Study groups and blood sampling

The study has been approved by the University Ethics Comission. After obtaining informed consent peripheral vein blood samples were drawn from 8 healthy parturients in active spontaneous labor with no previous medication and before obtaining any form of labor analgesia.

Five healthy non-pregnant women (students and staff) served as the control group.

Blood was sampled into 2.7 ml-EDTA tubes (Monovette, Sarstedt).

Luminol-dependent whole blood chemiluminescence (CL) according to Slavikova et al. [16]

200 μl samples of anticoagulated whole blood were incubated with 100 μl of different concentrations of bupivacaine in PBS or PBS alone (control samples) at 37°C for 10 minutes. End bupivacaine concentrations were 0.3, 30, and 3000 μM. Then 100 μl 1 mM luminol solution and 100 μl of opsonized zymosan (OZ) 10 mg mL^{-1} (end concentration 1 mg mL^{-1}) for stimulated samples were added. The total volume of 1000 μl was reached by adding PBS. The assays were run in duplicates. CL signals were recorded every 5 minutes over a period of 60 minutes. CL values are expressed as the area under the time-activity curve (Vs).

Additionally, the end results are also expressed as a percentage of the control response (CL of OZ stimulated blood – CL of intact blood; at presence of drug-free solution).

Leukocyte and polymorphonuclear cell count in peripheral blood

The cells were counted in Buerker counting chamber.

Statistical evaluation

Results are expressed as the mean (M) ± standard error of the mean (SEM).

Differences between the applied bupivacaine concentrations were assessed by Friedman test, data between groups were compared by Mann-Whitney U-test. A value of $P < 0.05$ was considered statistically significant.

Table I. Peripheral blood leukocyte and polymorphonuclear cell (PMN) counts in parturients and non-pregnant women.

	Leukocytes [10^9 L^{-1}]	PMN [10^9 L^{-1}]	% PMN
Parturients (n=8)	9.7 ± 1.4#	7.7 ± 1.2#	79.8 ± 3.3o
Non-Pregnant (n=5)	5.3 ± 1.4	3.5 ± 1.5	63.8 ± 15.0

Part. vs. Non-Pregn. U Mann-Whitney, # - denotes P=0.002; o - denotes P=0.019

Results

In laboring women a significant leukocytosis and an increase in polymorphonuclear cell counts were observed (Table I.).

Bupivacaine at the applied concentrations failed to exert any significant effect on the whole intact blood CL of both parturients and non-pregnant women. At the highest concentration applied (3000 μM) the CL values were higher in parturients (Figure 1.).

In the OZ- stimulated blood bupivacaine at the concentration of 3000 μM significantly inhibited the CL values comparably in both groups (Figure 2, Table II.).

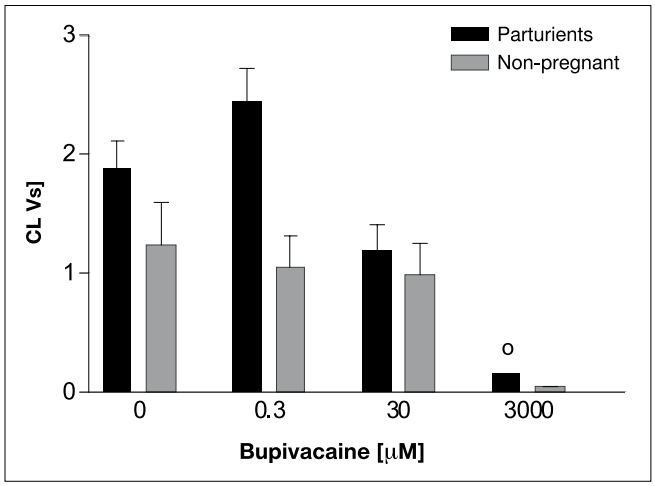
The net CL values, regarding both intact and OZ-stimulated whole blood, were slightly, insignificantly higher in laboring women (Figure1, 2). In order to avoid a bias in CL estimations due to an increased peripheral blood leukocyte and polymorphonuclear cell count in parturients (Table I) we also looked at the values expressed as a percentage of a control response. The obtained pattern was similarly comparable between laboring and non-pregnant women with the significant inhibition at the bupivacaine concentration of 3000 μM (Table II). The CL values expressed as a percentage of the control were significantly lower than the controls and the values obtained at 0.3 and 30 μM bupivacaine in laboring women. In the non-pregnant women significant differences between control and 0.3 μM bupivacaine concentrations as compared to 3000 μM were stated (Table II).

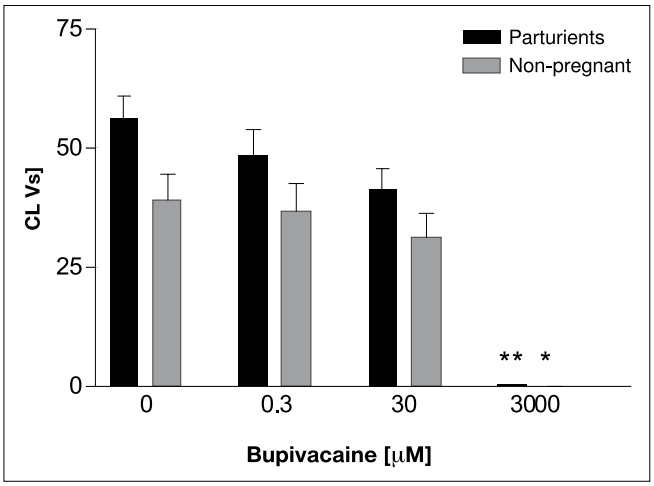
Discussion

In this study we have shown that bupivacaine depresses OZ-stimulated whole blood CL reflecting phagocytosis only at the highest applied, clinically irrelevant concentration (3000μM), in parturients being comparable to that observed in healthy young women (Figure 2, Table I). Bupivacaine did not significantly modify unstimulated whole blood CL; however, in parturients the values obtained by the bupivacaine concentration of 3000μM appeared to be higher than in non-pregnant controls. The net CL values, regarding both intact and OZ-stimulated whole blood, were slightly, insignificantly higher in laboring women (Figure 1, 2). However, we were also able to confirm an increased peripheral blood leukocyte and polymorphonuclear cell count in laboring women (Table I) and the phenomenon could influence the results obtained [17, 18]. Therefore, in order to avoid a bias in CL estimations connected with leukocytosis in parturients we also looked at the values expressed as a percentage of a control response. The obtained pattern was similarly comparable between laboring and non-pregnant women with the significant inhibition at the bupivacaine concentration of 3000 μM (Table II). This would indicate that the influence of bupivacaine at clinically encountered concentrations during labor (0.3-3 μM) on the whole blood ROS production coupled with phagocytosis could be neglected.

Chemiluminescence is an indirect measure of the phagocytic activity reflecting the ROS production coupled to phagocytosis and being proportional to this process [19]. In this study, to assess the effects of bupivacaine on the phagocytosis-associated CL by peripheral blood cells we applied a whole blood CL method, well accepted and having the advantage of a natural cell environment. It also allows avoiding isolation procedures which may cause cell activation, however at cost of lower sensitivity (quenching effects of erythrocytes and proteins) and specifity (contribution of various cells). Nevertheless, it may be assumed that the end product reflects mainly the oxidative activity of blood neutrophils [20]. On the other hand, if influence of a drug is tested with the use of a whole blood method, other problems that arise, like protein binding and indirect interactions effect, should be taken into consideration. However, information obtained, albeit relatively unspecific, better reflects the net biological product, important for clinical implications.

Our results are in line with most *in vitro* studies documenting in healthy donors the inhibitory effect of bupivacaine on the ROS production by phagocytic blood cells, i.e. neutrophils, with a concomitant decrease of phagocytosis and the expression impairment of surface receptors involved in this process (FcγRIII (CD16), CR1 (CD35), and CR3 (CD11b/CD18) [6, 8, 10]. ROS inhibition by LA was suggested to be associated with their physicochemical properties, specifically lipid solubility [7] and to be time-dependent [13]. Interestingly, the enantiomer-specific effects of bupivacaine concerning its influence on the ROS production were also noted, the S-(-) enantiomer displaying significantly less inhibition [8]. On the other hand, S-(-) bupivacaine appeared to be more effective in suppressing the neutrophil priming [9]. Other authors denied any significant influence of bupivacaine on cellular oxidative phenomena, chemotaxis, phagocytosis, intracellular calcium concentration and protein kinase activity and also neglected its effect on the ROS production in a non-cellular setting (xanthine-xanthine oxidase) [11, 12, 14].





Bupivacaine concentration [μM]	0	0.3	30	3000
Parturients (n=8)	1.87 ± 0.24	2.43 ± 0.28	1.18 ± 0.23	0.15 ± 0.01°
Non-Pregnant (n=5)	1.23 ± 0.37	1.05 ± 0.26	0.98 ± 0.27	0.04 ± 0.01

° - denotes Part. vs. Non-Pregn., U Mann-Whitney, P=0.034. In either group no significant differences between the concentrations analyzed were noted.

Figure 1. Whole blood chemiluminescence (CL) of parturients and non-pregnant women [Vs]; intact blood incubated with increasing bupivacaine concentrations; M ± SEM.

Bupivacaine concentration [μM]	0	0.3	30	3000
Parturients (n=8)	56.22 ± 4.59	48.27 ± 5.40	41.17 ± 4.64	0.14 ± 0.01**
Non-Pregnant (n=5)	39.00 ± 5.67	36.48 ± 6.24	31.18 ± 5.02	0.04 ± 0.01*

** - denotes 0 vs. 3000 μM bupivacaine, Friedman, P=0.0001,
* - denotes 0 vs. 3000 μM bupivacaine, Friedman, P=0.014. Between the groups no differences could be found

Figure 2. Whole blood chemiluminescence (CL) of parturients and non-pregnant women [Vs]; OZ stimulated blood incubated with increasing bupivacaine concentrations; M ± SEM.

It should be stressed that our and previously reported data describe inhibitory effects of bupivacaine on phagocytosis and the ROS production at high, clinically irrelevant concentrations. The half maximal inhibitory concentration (IC_{50}) reflecting an inhibitory potential of bupivacaine on the ROS production in isolated phorbol ester-stimulated neutrophils was estimated to be of 1.62 ± 0.05 mM [7]. Kiefer et al. investigating the influence of bupivacaine on the neutrophil respiratory burst accompanying phagocytosis could observe a relevant inhibition at the concentration of 770 μM. Interestingly, other amide local anesthetic, lidocaine, also occasionally applied in obstetric anesthesia was shown to be able to block OZ stimulated blood neutrophils already in therapeutic doses [21], whereas ropivacaine, a an S-(-)-enantiomer gaining popularity in obstetrics due to lower than bupivacaine cardio- and neurotoxicity seemed not to exert any relevant effect even in high concentrations [7].

Conclusions

Bupivacaine suppresses CL of zymosan stimulated whole blood of healthy parturients in a concentration-dependent manner, comparably to non-pregnant women. The effect is less expressed but more significant in laboring women, however negligible at clinically relevant concentrations in both groups.

Table II. The influence of bupivacaine on OZ stimulated whole blood CL response in parturients and non-pregnant women - % of control values; M ± SEM

Bupivacaine concentration [μM]	0	0.3	30	3000
Parturients (n=8)	100	93.91± 130.81	91.62 ± 110.46	0.64 ± 2.23***,*
Non-Pregnant (n=5)	100	81.07 ± 31.22	69.69 ± 29.80	0.01 ± 0.04**

Parturients: *** P< 0.001: 0-3000 μM bupivacaine, * P< 0.05: 0.3 – 3000 and 30 -3000 μM bupivacaine
Non-Pregnant: ** P < 0.01: 0-3000 i 0.3 -3000 μM bupivacaine
No significant differences between the groups.

References

1. Molloy E, O'Neill A, Grantham J, [et al.]. Labor induces a maternal inflammatory response syndrome. *Am J Obstet Gynecol.* 2004, 190, 448-455.
2. Fainaru O, Almog B, Pinchuk I, [et al.]. Active labour is associated with increased oxidisibility of serum lipids ex vivo. *BJOG.* 2002, 109, 938-941.
3. Yuan M, Jordan F, McInnes I, [et al.]. Leukocytes are primed in peripheral blood for activation during term and preterm labour. *Mol Hum Reprod.* 2009, 15, 713-724.
4. Cassuto J, Sinclair R, Bonderovic M. Anti-inflammatory properties of local anesthetics and their present and potential clinical implications. *Acta Anaesthesiol Scand.* 2006, 50, 265-282.
5. Flannagan R, Jaumouill´e V, Grinstein S. The cell biology of phagocytosis. *Annu Rev Pathol.* 2012, 7, 61–98.
6. Cederholm I, Briheim G, Rutberg H, [et al.]. Effects on five amino-amide local anesthetic agents on human polymorphonuclear leucocytes measured by chemiluminescence. *Acta Anaestesiol Scand.* 1994, 38, 704-710.
7. Hattori M, Dohi S, Nozaki M, [et al.]. The inhibitory effects of local anesthetics on superoxide generation of neutrophils correlate with their partition coefficients. *Anesth Analg.* 1997, 84, 405-412.
8. Welters I, Menzebach A, Langefeld W, [et al.]. Inhibitory effects of S-(-) and R-(+) bupivacaine on neutrophil function. *Acta Anaesthesiol Scand.* 2001, 45, 570-575.
9. Hollmann M, Kurz K, Herroeder S, [et al.]. The effects of s(-)-, r(+)-, and racemic bupivacaine on lysophosphatidate-induced priming of human neutrophils. *Anesth Analg.* 2003, 97, 1053-1058.
10. Kiefer R, Ploppa A, Krueger W, [et al.]. Local anesthetics impair human granulocyte phagocytosis activity, oxydative burst, and CD11b expression In response to Staphylococcus aureus. *Anestesiology.* 2003, 98, 842-848.
11. Mikawa K, Akamatsu H, Nishina K, [et al.]. Inhibitory effects of local anesthetics on reactive oxygen species production by human neutrophils. *Acta Anaestesiol Scand.* 1997, 41, 524-528.
12. Mikawa K, Akamatsu H, Nishina K, [et al.]. Effects of ropivacaine on human neutrophil function: comparison with bupivacaine and lidocaine. *Eur J Anaesthesiol.* 2003, 20, 104-110.
13. Ploppa A, Kiefer R, Krueger W, [et al.]. Local anesthetics time-dependently inhibit staphylococcus aureus phagocytosis, oxidative burst and CD11b expression by human neutrophils. *Reg Anesth Pain Med.* 2008, 33, 297-303.
14. Kawasaki C, Kawasaki T, Ogata M, [et al.]. Lidocaine enhances apoptosis and suppresses mitochondrial functions of human neutrophil in vitro. *J Trauma.* 2010, 68, 401-408.
15. Labedzka M, Gulyas H, Schmidt N, Gercken G. Toxicity of metallic ions and oxides to rabbit alveolar macrophages. *Environ Res.* 1989, 48, 255-274.
16. Slavikova H, Lojek A, Hamar J, [et al.]. Total antioxidant capacity of serum increased in early but not late period after intestinal ischemia in rats. *Free Radic Biol Med.* 1998, 25, 9-18.
17. Acker D, Johnson M, Sachs B, Friedman E. The leukocyte count in labor. *Am J Obstet Gynecol.* 1985,153, 737-739.
18. Preisler M, Weber P, Tempelman R, [et al.]. Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. *Am J Vet Res.* 2000, 61, 14-19.
19. Volle J, Tolleshaug H, Berg T. Phagocytosis and chemiluminescence response of granulocytes to monodisperse latex particles of varying sizes and surface coats. *Inflammation.* 2000, 24, 571-582.
20. Kopprasch S, Graessler J, Kohl M, [et al.]. Comparison of circulating phagocyte oxidative activity measured by chemiluminescence in whole blood and isolated polymorphonuclear leukocytes. *Clin Chem Acta.* 1996, 253,145-157.
21. Arakawa K, Takahashi H, Nakagawa S, [et al.]. The effects of lidocaine on superoxide production and p47 phox translocation in opsonized zymosan-activated neutrophils. *Anesth Analg.* 2001; 93, 1501–1506.