

THE INFLUENCE OF BUPIVACAINE ON THE CORD BLOOD CHEMILUMINESCENCE IN FULL TERM INFANTS – A PRELIMINARY REPORT

WPLYW BUPIWAKAINY NA CHEMILUMINESCENCJĘ KRWI PĘPOWINOWEJ NOWORODKÓW DONOSZONYCH – DONIESIENIE WSTĘPNE

¹Experimental Anaesthesiology Department, Poznan University of Medical Sciences, Poland
Head: Hanna Billert, MD

²Clinics of Perinatology and Gynecology, Poznan University of Medical Sciences, Poland
Head: Grzegorz H. Bręborowicz, MD, PhD

³Clinics of Obstetrical and Gynecological Anaesthesiology, Poznan University of Medical Sciences, Poland
Head: Michał Gaca, MD, PhD

Summary

Introduction. It has been shown that neutrophil function in the neonate may be modified according to mode of delivery and analgesia or anaesthesia technique applied to the mother.

Aim. The purpose of this study was to assess the influence of bupivacaine on the reactive oxygen species (ROS) production by neutrophils in the umbilical cord blood.

Methods. Luminol-dependent opsonized zymosan stimulated whole blood chemiluminescence (CL) was measured in umbilical vein blood samples drawn from six full term healthy neonates incubated with 0.1, 10, 1000 μM of bupivacaine. Simultaneously, peripheral blood CL of six healthy volunteers who served as the control group was also assessed. In the cord blood no significant changes were noted at all concentrations measured. A significant CL decrease at the concentration of 1000 μM could be observed in the control group.

Conclusion. Bupivacaine does not significantly influence opsonized zymosan stimulated luminol dependent whole cord blood CL *in vitro* at all concentrations measured. The question of possible implications of different reactivity of foetus and neonate to local anaesthetics for alternative effects of these compounds under physiological and pathological conditions warrants further studies.

KEY WORDS: cord blood, bupivacaine, chemiluminescence.

Streszczenie

Wstęp. Sposób ukończenia porodu i technika analgezji lub znieczulenia mogą wpływać modulująco na czynność granulocytów obojętnochłonnych noworodka.

Cel. Celem niniejszej pracy była ocena wpływu bupiwakainy na produkcję reaktywnych form tlenu (RFT) przez granulocyty obojętnochłonne krwi pępowinowej stymulowane opsonizowanym zymosanem.

Metodyka. Ocenie poddano zależną od luminolu chemiluminescencję (CL) krwi pełnej pępowinowej pobranej od sześciu urodzonych o czasie zdrowych noworodków inkubowanej z bupiwakainą w stężeniach 0,1, 10, 1000 μM . Jednocześnie badano CL krwi obwodowej pobranej od sześciorga zdrowych ochotników, którzy stanowili grupę kontrolną. Nie stwierdzono istotnych zmian CL krwi pępowinowej pod wpływem żadnego z badanych stężeń środka, podczas gdy w grupie kontrolnej zaobserwowano istotny wpływ hamujący bupiwakainy w stężeniu 1000 μM .

Wniosek. Bupiwakaina w żadnym z badanych stężeń nie wpływa istotnie na zależną od luminolu CL krwi pełnej pępowinowej *in vitro*. Zagadnienie ewentualnych implikacji odrębnej reaktywności granulocytów obojętnochłonnych płodu i noworodka na anestetyki lokalne w aspekcie efektów alternatywnych tych środków w warunkach fizjologicznych i patologicznych wymaga dalszych badań.

SŁOWA KLUCZOWE: krew pępowinowa, bupiwakaina, chemiluminescencja.

Background

After delivery host resistance mechanisms are crucial for the adaptation of neonates to changed environmental conditions. Spontaneous term labor is associated with an upregulated neonatal inflammatory response [1]. Fetal and neonatal peripheral blood leukocytosis is due to elevated counts of neutrophils, monocytes and natural killer cells [2]. Phenotypic and functional characteristics of neutrophils which play an important role in phagocytosis and are a key source of reactive oxygen species (ROS) exhibit a number

of differences as compared to adults [3]. Recently, increased activation of nuclear factor- κB (NF- κB) in newborn neutrophils has been revealed [4]. The neutrophil respiratory burst activity is comparable to that of adults, despite an altered kinetics [3, 5].

Interestingly, it has been shown that neutrophil function may be modified according to mode of delivery and analgesia or anaesthesia technique applied to the mother; however, reported results are conflicting [6, 7]. In the literature a necessity of continuing research on clinical neonatal effects of drugs being administered to the mother is frequently being emphasized [8].

Regional analgesia and anaesthesia techniques, considered to be methods of choice in obstetrics, proved beneficial for both mother and foetus. They allow minimizing undesired exposure to opioids and improve placental perfusion and oxygenation of the foetus [9]. However, some issues, i.e. concerning maternal fever and neonatal sepsis evaluation, remain controversial [8, 10]. During anaesthetic procedures local anaesthetics (LA) penetrate from maternal into foetal circulation resulting in foetal direct exposure [11]. A question arises whether LA are able to modulate foetal and neonatal host resistance. In fact, it is possible that administered medications may influence foetal immune system both indirectly, i.e. influencing endocrine responses which are tightly integrated with host resistance, and directly, interacting with the inflammatory cells [12].

Properties of LA to modify some inflammatory processes, i.e. to inhibit leukocyte metabolism have been recognized for a long time [13, 14]. However, only recently these alternative effects are increasingly gaining interest due to their promising therapeutic applications. In view of recent studies the impact on inflammatory cells is attributable to the interaction of LA with Gq regulatory proteins [15]. A number of newer studies addressed the issue of LA influence on the ROS production by neutrophils isolated from healthy adult volunteer peripheral blood [16, 17, 18, 19, 20]. Whether LA would display any alternative effects and influence foetal blood cells, both morphologically and functionally distinct from those of adults, has not been clearly elucidated.

Bupivacaine, an amide local anaesthetic agent belongs to one of the most commonly used LA in obstetrics [21]. Most investigators have shown its inhibitory effect in regard to leukocyte respiratory burst in adult healthy donors [18, 19, 22], however the published results are conflicting [20].

Whether bupivacaine would be able to modulate respiratory burst of cord blood cells is not known. The purpose of this study was to assess the influence of bupivacaine on the reactive oxygen species (ROS) production by neutrophils in the umbilical cord blood.

Material and methods

Reagents

Bupivacaine hydrochloride, luminol, 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. [23], a stock solution concentration was 10 mg mL⁻¹. Luminol was dissolved in borate buffer (pH 10), to obtain final concentration of 1 mM.

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

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

The study has been approved by the University Ethics Commission.

Umbilical vein blood samples were drawn from 6 healthy neonates whose mothers had uncomplicated pregnancy, normal labor and delivery, and did not receive any form of regional analgesia.

Six healthy volunteers of either sex (students and staff) served as the control group.

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

Luminol-dependent whole blood chemiluminescence was tested according to Slavikova et al. (24).

Two hundred 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. Final bupivacaine concentrations were 0.1, 10, 1000 µM. The lowest applied concentration was clinically relevant.

Then, 100 µl 1 mM luminol solution and 100 µl of opsonized zymosan 10 mg mL⁻¹ (final 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. Chemiluminescence was measured every 5 minutes over a period of 60 minutes. CL values were expressed as the area under the time-activity curve (Vs). The results were expressed as a percentage of the control response (CL of OZ stimulated blood – CL of intact blood; in presence of drug-free solution).

Statistical analysis

Data are represented as mean ± SEM. In order to detect differences between the applied bupivacaine concentrations Friedman test was applied, data between groups were compared by Mann-Whitney U-test. A value of P < 0.05 was considered statistically significant.

Results

Luminol dependent whole blood chemiluminescence values, in regard to both intact and OZ stimulated blood were slightly lower in cord blood specimens than those of adults, however the differences did not reach statistical significance (Table 1).

Table 1. Luminol dependent whole blood chemiluminescence (CL) values [Vs], intact and OZ-stimulated in the umbilical cord blood and control adults; mean ± SEM

CL [Vs]	Intact blood	OZ-stimulated blood
Neonates (n = 6)	0.5 ± 0.3	62.4 ± 12.5
Adults (n = 6)	3.6 ± 2.7	100.5 ± 45.2

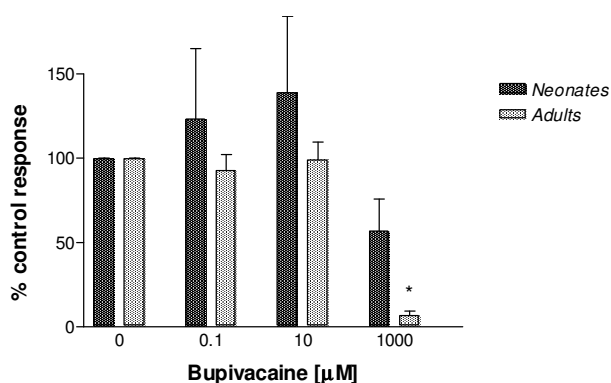
No significant differences between the groups were noted. Intact blood: P = 0.1274, OZ-stimulated blood: P = 0.8182, Mann-Whitney U-test

The influence of bupivacaine on the OZ-stimulated respiratory burst as expressed as % of control response is presented in Figure 1. A significant decrease at the concentration of 1000 µM could be observed in the control

group, in the cord blood no significant changes could be noted at all concentrations measured. In fact, in infants the observed pharmacodynamic profile followed a bi-phasic pattern with an increase of chemiluminescence at lower (clinically relevant) concentrations; however, these changes failed to reach a statistical significance.

Between the groups no significant changes could be detected at all concentrations measured.

Figure 1. Opsonized zymosan (OZ) stimulated whole blood chemiluminescence in the presence of increasing bupivacaine concentrations [% of control response]; umbilical vein and venous adult blood; mean \pm SEM



Bupivacaine concentration [µM]	0.1	10	1000
Neonates (n = 6)	123.0 \pm 42.1	139.1 \pm 45.2	56.5 \pm 19.2
Adults (n = 6)	92.9 \pm 9.4	99.2 \pm 10.4	6.7 \pm 2.6*

* – 1000 vs. 0 µM bupivacaine, Friedmann, $P < 0.05$; neonates vs. adults, U Mann-Whitney, n.s.

Discussion

In this study we have demonstrated that bupivacaine does not exert any significant influence on the cord whole blood respiratory burst due to the opsonized zymosan (OZ) stimulation (Figure 1).

At lower concentrations applied, the agent even displayed stimulatory effects towards cord blood cells, which however failed to reach a statistical significance.

In the adult controls a significant inhibition could be observed at the bupivacaine concentration of 1mM, which was in fact clinically irrelevant. However, despite different reaction pattern, we failed to prove any significant difference between foetal and adult whole blood chemiluminescence at the concentration of 1000 µM.

OZ-stimulated whole blood chemiluminescence as a measure of respiratory burst accompanying phagocytosis – methodological considerations

The method applied by us constitutes an accepted tool to assess respiratory burst of blood cells, and reflects mainly ROS neutrophil production [24]. OZ stimulation reflects the process connected with phagocytosis of

opsonized particles. Small amounts of samples and reagents required belong to main advantages of CL techniques, especially if whole blood is applied. Some interfering factors, as quench effects by erythrocytes and proteins, resulting in decreased sensitivity should be taken into consideration. Another problem is posed by limited specificity due to ROS production by not only neutrophils but also, even if with lower efficacy by monocytes, platelets and erythrocytes and also by intercellular interactions which contribute to the end product. However, a whole blood approach allows for better insight into the processes which take place in natural surrounding, so its clinical implications appear to be practically more relevant [25]. If whole blood CL method is applied to analyze an influence of a particular compound, like LA, questions regarding plasma binding capacity of the drug and its local availability might additionally influence the end result.

Reactivity of adult blood phagocytes to LA – respiratory burst and phagocytosis

Most anaesthetic agents, both general and local ones, were reported to be able to suppress neutrophil CL [16]. LA were demonstrated to inhibit respiratory burst due to phagocytosis. Lidocaine has been shown to inhibit OZ-induced p47 plox translocation, a subunit of NADPH oxidase [17]. Data on bupivacaine mostly confirm its inhibitory effects on ROS production [18, 19] which is consistent with our own observations regarding adult controls. It has been shown that the agent inhibits surface expression of the receptors taking part in the phagocytosis process of opsonized particles (FcγRIII, CR1 and CR3, 18). Kiefer et al. reported significant inhibitory effects of bupivacaine at the concentration of 770 µM (neutrophils in whole blood, flow cytometry) which could be considered as consistent with our observations [19]. Some other investigators did not observe any effect of bupivacaine on the respiratory burst and phagocytosis [20].

The influence of bupivacaine on the whole cord blood CL

In the umbilical cord blood we were not able to observe any significant effect of bupivacaine on the whole blood ROS production due to OZ-stimulation (Figure 1). The lowest concentration applied by us, 0.1 µM, approximates cord plasma levels in neonates whose mothers were administered epidural analgesia for labor pain relief. Levels in infants of mothers anaesthetized epidurally for cesarean section are about 10 times higher. Mean foetal/maternal total plasma concentration ratios for bupivacaine were reported to be about 0.3 [26, 11]. We could not observe any significant inhibitory effect of bupivacaine on the cord blood CL even at concentrations much higher as those clinically relevant (1000 µM). The observed by us resistance to an inhibitory effect of bupivacaine could be possibly explained by the influence of stress and systemic inflammatory mediators, i.e. cyto-

kines and/or may otherwise indicate differences between foetal and adult blood cells. Immaturity and a morphologically unique ultrastructure of cord blood cells could be demonstrated using electron microscopy [27]. Numerous studies have dealt with functional characteristics of foetal and neonatal phagocytes. Adinolfi et al. reported increased expression of receptor molecules participating in phagocytosis (CR1 and CR3) on the surface of monocytes and neutrophils from newborn (cord, and also maternal) blood samples as compared to adult controls [28]. Most investigators could not observe any differences in the neutrophil respiratory burst activity of healthy neonates as compared to that of adults [5]. Our data confirm no significant differences in both intact and OZ-stimulated CL values between neonates and control adults (Table 1). Some other investigators could observe higher ROS production of intact neutrophils and impaired phagocytosis in neonates as compared to adults [29]. On the other hand, according to published data, neonatal cells should display priming deficiency [30], and react differently to challenges such as sepsis or stress, lowering their ROS production capacity [3]. Our data suggest that as far as a respiratory burst due to phagocytosis of opsonized particles is considered, foetal and neonatal blood phagocytic cells may also react differently to pharmacological compounds than those of adult blood. Presumably the underlying mechanism could involve desensitization phenomena or/and an immaturity of G-protein coupled transduction pathways [31]; the issue however needs to be addressed by a more sophisticated approach.

ROS production, if excessive, may be harmful to the neonate underlying numerous pathological situations; however their beneficial influences, i.e. in regulating tissue growth, are recently frequently emphasized [32]. Therefore, therapeutic interventions which decrease oxidative processes in the neonate could be undesirable under physiological circumstances.

In conclusion, our data confirm the safety of labor local analgesia and anaesthesia for the foetus. However, the question of possible implications of different reactivity of foetus and neonate to LA as considered alternative effects of these compounds under both physiological and pathological conditions warrants further studies.

Conclusion

Bupivacaine does not influence opsonized zymosan stimulated luminol dependent whole cord blood chemiluminescence in vitro at all concentrations measured.

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