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Review Article

REDOX THERAPY IN NEONATAL SEPSIS: REASONS, TARGETS, STRATEGY, AND AGENTS

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ABSTRACT—Neonatal sepsis is one of the most fulminating conditions in neonatal intensive care units. Antipathogen and supportive care are administered routinely, but do not deliver satisfactory results. In addition, the efforts to treat neonatal sepsis with anti-inflammatory agents have generally shown to be futile. The accumulating data imply that intracellular redox changes intertwined into neonatal sepsis redox cycle represent the main cause of dysfunction of mitochondria and cells in neonatal sepsis. Our aim here is to support the new philosophy in neonatal sepsis treatment, which involves the integration of mechanisms that are responsible for cellular dysfunction and organ failure, the recognition of the most important targets, and the selection of safe agents that can stop the neonatal sepsis redox cycle by hitting the hot spots. Redox-active agents that could be beneficial for neonatal sepsis treatment according to these criteria include lactoferrin, interleukin 10, zinc and selenium supplements, ibuprofen, edaravone, and pentoxifylline.

KEYWORDS—Antioxidants, mitochondria, neonate, oxidative stress, sepsis

ABBREVIATIONS—GSH — glutathione; GPx — glutathione peroxidase; H_2O_2 — hydrogen peroxide; iNOS — inducible nitric oxide synthase; NF-κB — nuclear factor κB; NICU — neonatal intensive care unit; $^{\bullet}O_2^-$ — superoxide radical anion; $ONOO^-$ — peroxynitrite

INTRODUCTION

The treatment of critically ill neonates is particularly sensitive issue because neonatal sepsis is one of the most fulminating conditions in neonatal intensive care units (NICUs) that places an immense emotional burden and pressure on parents, physicians, and society. So, it is no wonder that current strategies in neonatal sepsis treatment are focused on pathogen eradication by an early application of empirical combination of narrow-spectrum antibiotics (1). Despite the guidelines, the reserve broad-spectrum antibiotics are often used for the empirical therapy of neonatal sepsis. Unfortunately, such approach delivers results that are far from satisfactory and encounters different setbacks perpetually. Broad-spectrum antibiotics exert selective pressure on specific bacterial species, which might promote the growth of some other bacteria or could leave ecological niche wide open for fungi. For example, the efforts to reduce the rate of group B streptococcus disease using intrepartum antimicrobial prophylaxis have been associated with increased rates of gram-negative infections (2). The incidence of neonatal sepsis caused by fungal infections has increased several folds in developed countries in the past two decades (3, 4), whereas the incidence of invasive candidiasis correlates with average per-infant use of broad-spectrum antibiotics (5). Furthermore, almost any NICU or country might find itself from time to time in a position to deal with the outbreaks of strains resistant to antibiotics that are routinely applied in accordance to the clinical practice or national authority's recommendations (6, 7). Antibiotic-resistant strains still represent a problem in low- and middle-income countries, related to the challenges of access (a limited number of antibiotics is available) and excess (the use of antibiotics is poorly controlled) (8). Finally, the regimens for neonatal sepsis treatment currently recommended by national pediatric associations do not adequately account for coagulase-negative *Staphylococcus* (9). This might be the cause of rising incidence of coagulase-negative *Staphylococcus*–caused neonatal sepsis in developed countries (3, 4).

Another general problem is the lack of clinical studies in neonates, which significantly affects efficacy, safety, and the quality of therapy. The majority of antibiotics and 90% of other drugs prescribed in NICUs are used in off-label and unlicensed manner (10, 11). The use of drugs based on extrapolated results obtained in infants, children, and adult population has generally been inadequate because of the significant pharmacokinetic and pharmacodynamic differences (12). The lack of pharmacokinetic and pharmacodynamic data for antibiotics used in neonates increases the risk of overdosing and subdosing, adverse effects and/or inefficacy, and rapid development of resistance (13, 14). Clinical assessments of the current antibiotics as well as the development of new drugs, designed on the basis of pathophysiological characteristics of disease in neonates, are imperative for efficient and safe therapy for neonatal sepsis (15, 16).

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NEONATAL SEPSIS REDOX CYCLE

In two recent editorials reflecting on yet another failure of an anti-inflammatory agent to treat sepsis, the authors concluded that "This setback should inspire a redoubling of efforts to seek new approaches to treatment that are based on a more crystalline view of the biology of sepsis" (17), and "In the end, however, a single cure for sepsis seems unlikely. A deeper understanding of the processes leading to sepsis is necessary before we can design an effective suite of interventions" (18). So, in parallel to day-to-day fighting, we ought to look at what has been done in the field of the pathophysiology of neonatal sepsis in order to explore new treatment possibilities. And this is by no means little, as the focus has moved considerably in the past decade from the immune system activity to intracellular redox changes. Previously accepted hypothesis that (neonatal) sepsis is in fact an uncontrolled inflammatory response (19) has faced the "wall" of unsuccessful trials (20, 21). It turned out that, during the course of sepsis progression, immune system shows not one but two drastically different phases: hyperactive and hypoactive (20). Furthermore, some phenomena, such as negative blood cultures, which are frequently confirmed in sepsis patients (22, 23), and sporadic cases of sepsis provoked by pathogen-unrelated insults (24), imply that, like inflammation, the infection itself might only represent a step in the initiation of sepsis mechanisms that thereafter act independently of inflammation or the type of pathogen and are ubiquitous to all sepsis patients. A new player emerged on the scene in 2001 (although it was first proposed in 1995 by Vlessis and colleagues [25]), when Fink (26) postulated that dysfunctional mitochondria and redox changes are to be blamed for organ failure in sepsis. Since then, accumulating data increased the awareness of scientific community that redox processes taking place in the intracellular compartment, and mitochondria in particular, have an essential role in the pathogenesis of sepsis (27–30). We have recently integrated the state-of-the-art knowledge on neonatal and adult sepsis in order to postulate that a self-sustaining and self-promoting (neonatal) "sepsis redox cycle" is initiated in the intracellular compartment (Fig. 1), resulting in mitochondria, cell, and eventually organ dysfunction (27, 28). In brief, the "cycle" in neonatal cells sets off with interleukins (IL-6 and IL-8)–mediated activation of nuclear factor κB (NF-κB), which regulates the expression of inducible nitric oxide (NO) synthase (iNOS). High concentrations of NO inhibit electron transport chain, resulting in increased production of superoxide radical anion ($^{\bullet}O_2^-$) and hydrogen peroxide (H_2O_2) in mitochondria (31, 32). Hydrogen peroxide from mitochondria further activates NF-κB, which results in the development of self-sustaining/promoting loop leading to mitochondria dysfunction, energy failure, and cellular dysfunction. The "cycle" is additionally supported by NF-κB–activated expression of cyclooxygenase 2, which has $^{\bullet}O_2^-$ as a by-product. Superoxide is dismutated to H_2O_2, both in mitochondria and cytoplasm. In mitochondria, $^{\bullet}O_2^-$ reacts with NO to produce peroxynitrite ($ONOO^-$). Peroxynitrite inhibits glutathione (GSH) reductase, which is coupled with GSH peroxidase (GPx), the key enzyme for H_2O_2 removal in mitochondria. In addition, $ONOO^-$ can be decomposed to two very harmful species—hydroxyl and nitrogen dioxide radical. NO and H_2O_2 can leak from the cell in order to activate neonatal sepsis redox cycle in surrounding tissue. There are several key differences in the redox signaling and redox-mediated cellular/tissue

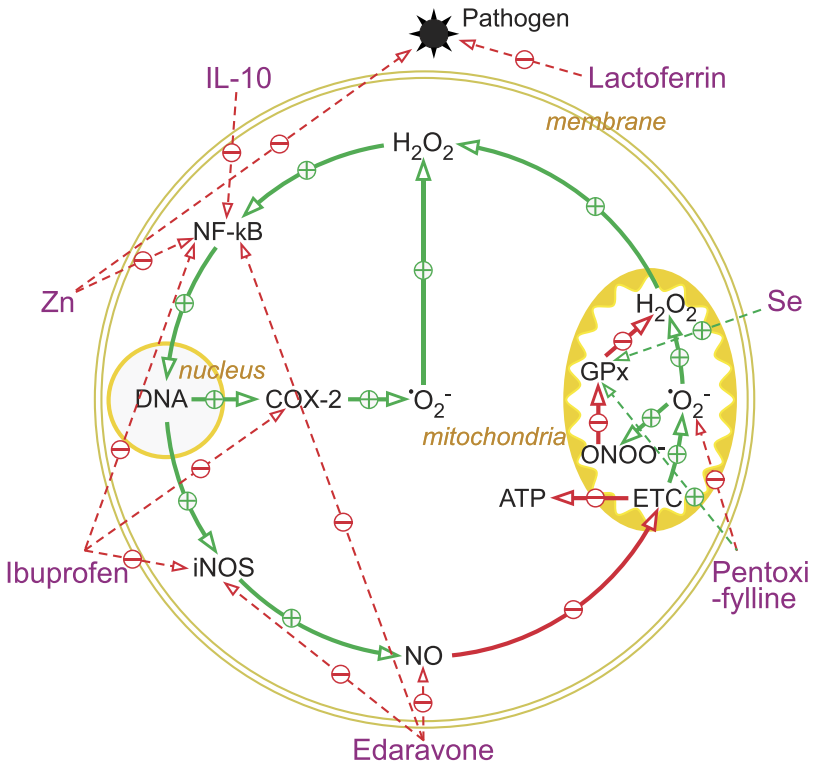

Fig. 1. **Neonatal sepsis redox cycle, redox-active agents and their targets in neonatal sepsis treatment**. Neonatal sepsis redox cycle is presented in full lines (+ promotion/increase; - inhibition/decrease). The effects of agents on specific targets in neonatal sepsis redox cycle are presented with dashed lines.

damage that clearly delineate neonatal sepsis as a separate entity from adult sepsis: (i) immature innate immune system in neonates shows low capacity to generate reactive oxygen species (33), so pro-oxidative processes in neonatal sepsis are generally limited to intracellular compartment of affected tissues. (ii) Neonatal cells regulate the level of H_2O_2 via GPx, whereas adult cells generally use catalase. As an illustration, GPx activity is twofold to sixfold higher, whereas catalase activity is threefold lower in neonatal tissues compared with adults (34). (iii) Neonatal cells appear to compensate for sepsis-provoked mitochondrial dysfunction by extramitochondrial ATP production (35), which is not the case in adult tissues (36). (iv) Proliferating cells are particularly susceptible to apoptosis that can be initiated by oxidative stress (28). In relation to this, neonatal sepsis-related cellular dysfunction might result in devastating effects in developing tissues, such as brain (periventricular leukomalacia, intraventricular hemorrhage, cerebral palsy, vision impairments), lungs (respiratory distress syndrome and bronchopulmonary dysplasia), and heart (patent ductus arteriosus) (37, 38). In contrast, adult sepsis provokes reversible changes or minor damage (39, 40). This explains a considerably higher incidence of long-term effects in neonatal sepsis survivors (41) compared with adults (42), but also points out the importance of redox therapy in neonatal sepsis treatment.

TARGETS AND STRATEGY OF REDOX THERAPY

Redox-active molecules form a complex, intertwined system in neonatal sepsis that cannot be repaired by a blunt application of unspecific antioxidants hoping that they will do well, which has often been the case in animal studies and even some clinical trials. This is probably one of the reasons why believable benefit from application of some antioxidants has not been translated into success in human clinical trials (43–45). Instead, redox approach should target specific steps in the pathogenesis of neonatal sepsis. A plausible agent should be capable of entering and accumulating in the intracellular compartment and mitochondria of affected tissues. This is not an easy task because cells comprise a refractory system in order to tightly control the intracellular level of reducing species (e.g., vitamins C and E), which is essential for maintaining a balanced redox poise and normal redox signaling (46). The application of preferentially extracellular redox-active agents might be futile, whereas prophylactic application of such agents could even show negative effects in neonates. Pertinent to the former, we have recently shown that redox settings in the blood of septic neonates are no different compared with matching controls (47). Based on this, it was proposed that the application of vitamin E in septic neonates might not be rational. Even more, one Cochrane study documented that prophylactic vitamin E significantly increases the risk of sepsis in neonates (48). This could be explained by the direct scavenging activity of vitamin E and its ability to increase the capacity of neonatal erythrocytes to remove reactive oxygen species, which are used by the innate immune system to kill pathogens (47). In this way, already weak neonatal immune system might be further compromised. However, some extracellular agents could still find a place in neonatal sepsis prophylaxis. The availability of iron plays an important role in sepsis because iron is obligatory for proliferation of bacteria. Hence, the sequestration of iron in plasma should decrease the number of pathogens (49). Iron-removing strategy might be particularly important in newborns, because their system of iron regulation is immature; i.e., they show low levels of transferrin and ceruloplasmin (50). Lactoferrin is the only iron-sequestering agent that is tested in clinical trials on neonates. One study on 472 very low-birth-weight neonates showed that lactoferrin substantially reduces the incidence of both bacterial and fungal sepsis (51), and there are several ongoing multicenter studies further examining this issue. It is worth mentioning that observational studies imply that breast milk, which contains lactoferrin and reduces the growth of *Escherichia coli* and other gram-negative pathogenic bacteria, has an impact on infection-specific mortality rates during neonatal period (52). We have described recently a unique antioxidative profile of breast milk, which might be relevant for its beneficial effects in neonates exposed to infection (53).

PROMISING REDOX-ACTIVE AGENTS

The strategy of drug development for the treatment of neonatal sepsis is based on the evaluation of the existing drugs and the development of new drugs for unique pathophysiological processes characteristic for neonates. Child health is a priority for European Commission (EC), as illustrated by the topic in the very first FP7 call—"Paediatric Medical Products: Adapting Off Patient Medicines to the Specific Needs of Paediatric Populations," as well as by TINN (Treat Infections in Neonates) programs, which are founded in order to evaluate anti-infective drugs (ciprofloxacin, fluconazole, and azithromycin) in neonates. These drugs are prescribed off-label to treat life-threatening neonatal infections. Apart from neonatal clinical drug evaluations, this research also contains *in silico* experiments and animal studies and evaluates formulations adapted to neonates. Modeled on these EC programs, the incitement of the neonatal evaluations of existing promising redox-active drugs, as well as the development of the new ones based on the knowledge of sepsis mechanisms including neonatal sepsis redox cycle, would certainly contribute to the significant improvement of therapy of neonatal sepsis.

The available data on redox therapy for neonatal sepsis are rather limited. For example, a brief systematic search of PubMed (electronic database of the US National Library of Medicine) for articles that contain the term *neonatal sepsis*, word *therapy* or *treatment*, and one of the following words: *redox*, *antioxidant*, and *antioxidative*, returned only some 100 results. Nevertheless, there is a small set of promising and generally safe agents that could be applied in the treatment of neonatal sepsis. Several agents can be used to suppress the first step in neonatal sepsis redox cycle: NF-κB–induced gene expression (Fig. 1). For example, Mittal and coworkers (54) have recently proposed that low-quantity IL-10 would be beneficial in the treatment of neonates with meningitis, based on the results showing that IL-10 prevents infection-related brain damage and decreases the mortality rate in model animals infected even with antibiotic-resistant strains. Interleukin 10 is known to inhibit NF-κB expression (55) and the transduction of extracellular inflammatory stimuli to intracellular production of reactive species (56). In a recent clinical trial, the supplementation of zinc (Zn) in infants aged between 7 and 120 days with probable serious bacterial infection resulted in

significantly fewer treatment failures compared with placebo group (57). Such positive effects could be at least partially attributed to Zn-provoked inhibition of NF-κB in vital organs (58, 59) and potentially to direct effects of Zn on some pathogens (60). It is worth mentioning that oral Zn supplementation given at high doses reduces morbidities (including sepsis-related) and mortality in preterm neonates (61). Ibuprofen decreased the level of IL-6 and C-reactive protein in septic neonates and attenuated lethality of neonatal sepsis in model animals (62). The beneficial effects of ibuprofen could be at least partially attributed to its capacity to abrogate NF-κB, iNOS, and cyclooxygenase 2 activity (63). It is noteworthy that ibuprofen represents a reasonably effective drug for patent ductus arteriosus closure in neonates (64). Edaravone (3-methyl-1-phenyl-pyrazolin-5-one), a relatively novel free radical scavenger on the market, has been shown to prolong the survival of septic newborn piglets (65). In sepsis models, edaravone inhibited the activation of NF-κB, reduced the induction of iNOS, and decreased NO production (65, 66). A more recent study found that edaravone might inhibit the induction of iNOS gene expression in an NF-κB–independent manner (67). There are no data on the safety of edaravone in neonates, but it has been approved by Japanese regulatory authorities for clinical use in the management of acute ischemic stroke. It should be stressed that NF-κB inhibitors generally should not be applied in the prophylaxis of neonatal sepsis, as they might suppress reactive species-generating activity of immune system cells (68), thus leaving neonates more susceptible to infections.

A novel approach in antioxidative therapy is to target oxidative changes in mitochondria in order to preserve/regain their function, which is disrupted in neonatal sepsis. Two principal strategies for antioxidative protection of mitochondria have been postulated by Galley (29): (i) the application of radical scavengers that are delivered specifically to mitochondria (lipophilic cations, Tempol-conjugates, specific bioactive peptides) and (ii) the amplification of endogenous mitochondrial antioxidative protection, for example, via pharmacological upregulation of the GSH system. There are two promising agents that could mediate beneficial effects in neonatal sepsis via the GSH system, whereas data on other approaches of mitochondria-targeting redox therapy in neonatal sepsis are still awaited. Two randomized controlled trials enrolling 140 neonates with suspected/confirmed sepsis showed that xanthine derivative, pentoxifylline, significantly reduces mortality rate (69). Pentoxifylline restores GSH level and preserves viability of mitochondria showing inhibited respiration (70). The other agent is selenium (Se), which is essential for *de novo* synthesis of GPx (71). A Cochrane study found that the supplementation of Se is associated with significant reduction of neonatal sepsis incidence (72). Premature newborns are often Se deficient, and early Se supplementation appears to be very important for preventing and treating sepsis in this group (73). There are some other strategies that could be tested on animal models for neonatal sepsis. Molecular hydrogen (H_2), a small gaseous reducing agent, is known to alleviate intracellular oxidative stress (74). The inhalation with 2% H_2 improved the survival rate of adult septic mice in a concentration- and time-dependent manner (75, 76). It appears important to examine a potential link between the ability of intestinal bacteria to produce H_2 (77) and the effects of probiotics on sepsis and some other conditions in neonates (78). Finally, intracellular antioxidative system can be upregulated by promoting nuclear factor E2–related factor (Nrf2)–dependent transcription of antioxidative enzymes: NAD(P)H:quinine oxidoreductase 1 and GSH-related enzymes. This can be achieved by the application of thiol-binding agents, which stabilize Nrf2 or modify the Nrf2 inhibitor, Kelch-like ECH-associated protein 1 (79). One agent that has the ability to activate Nrf2 and has been tested in adult sepsis is ethyl pyruvate (80). A potential problem with the application of this strategy in neonatal sepsis might be the suppression of the immune system via cell-surface thiol redox-switches modulation (81).

CONCLUSIONS

Neonatal sepsis still represents an immense problem in NICUs around the world, having the potential to escalate in the future because of the rise of antibiotic-resistant strains and fungal infections. Something has to be done to supplement current approach, save lives, and alleviate social and emotional burden. Our aim was to point out the new philosophy in neonatal sepsis treatment, which involves: (i) integrative approach on the mechanisms—neonatal sepsis redox cycle, resulting in cellular dysfunction and organ failure; (ii) recognition of the most important targets; and (iii) the selection of safe, low-cost agents that can stop the neonatal sepsis redox cycle. The redox therapy could show the best results if agents with different targets are applied in combination. As we noted previously, there is no "magic bullet" for sepsis treatment, but "magic bullets" are required instead. Further research and trials are needed, but it is important that we at least partially redirect our efforts toward testing available and developing new redox (antioxidative) agents in order to arrest the process of neonatal sepsis.

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