Pathophysiology of diseases in the newborn caused by reactive oxygen species (ROS)

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SUMMARY

The pathophysiological basis of oxidative stress is the disturbance of the delicate balance between oxidant production and destruction. As main oxidant producing systems in different organ are recognized: - iron overload and production of Fe^{++}; - the invasion of neutrophils and macrophages; - the cytochrome P450 system; the formation of hydroxyeicosatrienoic acids; - the NO system and the xanthine oxidase system during ischemia-reperfusion. All the mentioned pathophysiological events are common to the majority of the organ systems and represent the basis of clinical pathology. This pathophysiological understanding is the basis of specific therapeutic intervention.

Key words: Oxidative stress, glutathione, iron overload, oxidative burst, cytochrome P450, xanthine oxidase.

An increasing amount of evidence is demonstrating a clear relationship between different levels of oxidative stress and the development of disease in major organ systems during early infancy. Oxidative stress occurs when the delicate balance between oxidant production and destruction is disturbed within cells. When the rate of oxidant production exceeds the anti-oxidant capacity, reactive oxygen species (ROS) - mediated cell injury will occur. The oxidative reactivity increases from superoxide ($O_2^{\cdot -}$), via hydrogen peroxide (H_2O_2) to the hydroxyl radical ($\cdot OH$)[1]. As a group, most ROS are extremely potent oxidants and can inactivate DNA, proteins, carbohydrates and lipids. Although ROS production is discussed in the context of acute organ injury, it is important to remember that ROS are produced during many physiologic redox cycling reactions as for instance the killing of microorganisms by granulocytes and macrophages that requires a burst of oxidative activity[2]. Their form of clinical presentation depends on the organ involved and on a the specific distribution of oxidation promoting and inhibiting factors in that tissue. In the newborn period brain, lungs, gut and liver are the most prominent organ systems that will be adressed with respect to their pathophysiological aspects.

The lungs and respiratory therapy

The newborn lung is particularly susceptible to oxidant stress because there are many sources of ROS production and a relative lack of anti-oxidant defences. There is evidence that at least in premature infants lung anti-oxidant enzyme content is low and only slowly inducible by oxidant stress and that in addition GSH/GSSG recycling may be abnormal[3]. The glutathione content of lung epithelial cells is up to 100 times greater than in the plasma and this is reflected in high levels in alveolar epithelial lining fluid which may represent the first line of defense against oxygen[4]. The effectivity of the glutathione system depends on various cofactors:

1. The availability of cysteine and glutamine, the amino acids limiting for glutathione synthesis. The poor solubility of cysteine makes it a problematic amino acid during parenteral nutrition of the young infant. The impaired availability of glutamine mainly during periods of severe infections was realized only during the last few years.

2. Glutathione peroxidase depends on the availability of selenium.

3. GSSG is reconverted to GSH in a NADPH and riboflavin dependent reaction. Riboflavin guarantees the normal cellular relation between reduced (GSH) and oxidized glutathione (GSSG) which is about 100 to 400: 1. The most important aspect of riboflavin stability is its sensitivity to light, as it is readily decomposed for instance during phototherapy of the newborn[5]. The decreasing effect on glutathione reductase as well as on xanthine oxidase

activities could be demonstrated especially when phototherapy lasted longer than 100 hours[6]. A decreased serum uric acid concentration is another effect of lacking riboflavin on the xanthin oxidase activity[6]. As it is widely accepted that uric acid is an effective antioxidant in human plasma, low riboflavin concentrations indicate an insufficient antioxidative protection via insufficient glutathione regeneration and uric acid formation.

ROS production is increased by acute hyperoxia mainly during artificial ventilation, asphyxia, cycles of ischemia and reperfusion, sequestration and activation of lung macrophages and granulocytes and by lung infection and inflammation.

In vivo the pulmonary microvascular endothelial cell is especially vulnerable. Accelerated ROS production can inactivate surfactant[7] and lung anti-proteases[8] and increase the production of pro-inflammatory compounds[9].

Lung cells contain at least seven P450 isoenzymes, mainly in endothelial cells, type II cells and the nonciliated bronchiolar epithelial Clara cells[10]. Cytochrome P450 isoenzymes can produce ROS either by uncoupling or by contributing haem-derived iron for Fenton reactions. In addition, new metabolites of arachidonic acid metabolism have been discovered recently that are produced by cytochrome P450 isoenzymes. They are mainly represented by the hydroxylation products, the hydroxyeicosatrienoic acids or "HETEs"[11]. These metabolites accumulate in the lungs of oxygen-exposed infants[12]. Evidence that oxidant stress causes pathology is often only indirect, obtainable by the determination of candidate markers. As such could serve tracheal aspirate levels of oxidized proteins such as nitrotyrosines[13].

Nitric oxide (NO) is a free radical gas that functions both as a signaling molecule in endothelial cells and the brain and as a „killer molecule" in activated immune cells. In an extremely rapid reaction between NO and superoxide (O.-) peroxinitrite is formed. Nitration of proteins attributed to peroxinitrite has been detected in the brain and at sites of inflammatory tissue injury, including the lungs of infants with bronchopulmonary dysplasia[14].

The brain and special aspects of iron metabolism.

Iron is the most important transition metal involved in the production of free oxygen radicals. Certain regions of the brain are particularly high in iron, such as the globus pallidus, the substantia nigra and the red nucleus. Free Fe^{++} ions react with H_2O_2 to form $OH^{\cdot}$ by the so called Fenton reaction, which is usually written as:

$Fe^{++} + H_2O_2 \dashrightarrow Fe^{+++} + OH^{\cdot} + OH^-$

Reducing agents, like ascorbate, stimulate Fe^{++} formation from Fe^{+++}; hence iron salt - ascorbate mixtures are good sources of hydroxyl radical ($OH^{\cdot}$) formation. Superoxide ($O2^{\cdot -}$) can reduce certain ferric chelates (Fe^{+++}) leading to the following final overall reaction:

$O_2^{\cdot -} + H_2O_2 \text{--Fe--}\dashrightarrow OH^{\cdot} + OH^- + O_2$

This reaction is called the iron-catalyzed Haber-Weiss reaction, or sometimes the superoxide-driven Fenton reaction.

Oxidative stress can itself provide the iron necessary for Fenton chemistry, for example by mobilizing iron from ferritin (via $O_2^{\cdot -}$) or by degrading heme protein to release iron (via H_2O^2).

At birth, plasma transferrin is lower and much more highly loaded with iron than in later life[15]. Leakage of plasma containing highly iron-loaded transferrin into the alveolar space may provide a source of iron that can initiate lipid peroxidation of pulmonary surfactant. Plasma from preterm babies has been demonstrated to induce iron-catalyzed lipid peroxidation of pulmonary surfactant in an in vitro model[16]. Non-protein-bound iron has been measured in the plasma of both preterm and term babies. High vitamin C levels in cord blood can reduce ferric iron (Fe^{+++}) and thereby antagonize the ferroxidase activity of ceruloplasmin[17]. A recent observation published by Silvers et al. is based on this interrelation, demonstrating a worse outcome in premature infants with high plasma vitamin C concentrations at birth[18]. The small amount of iron in maternal milk combinded with vitamin C enrichment in breast milk may reflect evolutionary wisdom with respect to the threat of hydroxyl radical production. Ceruloplasmin is also decreased in cord blood and the occurence of ferrous iron (Fe^{++}) has been demonstrated[19]. Ceruloplasmin is not only the major copper transporting serum protein but

also acts as a ferroxidase. This protein plays a direct role in the mobilization of iron from parenchymal tissues and the subsequent oxidation and incorporation of ferric iron into circulating apotransferrin.

Some inborn diseases clearly underline the oxygen radical producing effect of unbound ferrous ions. These are: perinatal hemochromatosis as a cause of neonatal hepatic failure, Wilsons disease and aceruloplasminemia. Although a high content of iron may be essential for brain function, its presence means that any injury to brain cells can result in release of iron ions that stimulate free-radical reactions. In addition there is a high concentration of ascorbic acid in gray and white matter of the CNS. The choroid plexus has a specific active transport system that raises ascorbate concentrations in the cerebrospinal fluid to greater than the plasma level[20].

In a discussion on the pathophysiology of oxidative stress in the central nervous system our recent knowledge concerning N-methyl-D-aspartate (NMDA) receptors has to be included. Excitatory amino acids such as glutamate and aspartate play a role in hypoxic brain damage. Activation of the NMDA-receptors has been shown to occur in the hypoxic brain[21]. An increase in glutamate has been shown to cause an increase in lipid peroxidation products, a decrease in the anti-oxidant enzyme catalase, and to significantly deplete total and free sulfhydryl groups in neonatal rat brain. Blocking the NMDA subclass of glutamate receptors may prevent the influx of calcium which leads to neuronal injury following ischemia.

The eye and retinopathy of prematurity (ROP)

ROP is an oxidative injury caused primarily by high inspired O_2 concentrations. The retina is especially prone to oxidative damage because it is well vascularized and has a high oxidative metabolic rate. The polyunsaturated fatty acid content in phospholipids of the outer rod membrane is at least 65 %, which is more than in any other tissue. ROP, recognized since the early 1940s, continues to be a cause of serious visual morbidity mainly in very-low-birth-weight children. The susceptibility to develope ROP is also influenced by the intensity of retinal melanin pigmentation. Melanines are photoprotective redoxpolymers acting as pseudosuper-oxide dismutase. This explains the fact that pigmented infants are better protected against oxygen radicals than less pigmented ones.

Under pathophysiological aspects, the influence of free iron on the generation of reactive oxygen radicals also has to be discussed. Blood transfusions could be related to a higher frequency of ROP. As hypothesised by Sullivan in 1988[22], blood transfusions increase the amount of free iron and lead to the generation of highly reactive oxygen radicals. Preterm infants are particularly susceptible to such iron overload because they receive frequent transfusions (about 3.7 ± 5.5 transfusions between admission and discharge)[23] which carry a significant iron load.

Under these pathophysiological insights iron-chelation therapy should be considered as one possible mechanism to inhibit free oxygen radical generated pathology. In this context the possibly preventive role of iron-free transferrin should be reviewed.

Necrotizing enterocolitis (NEC) and the problem of reperfusion

The disease represents the clinical manifestation of a complex pathophysiologic process that most importantly involves among others a hypoxia-ischemia-reperfusion injury. During periods of neonatal hypoxemic-ischemic stress an acute reflex diminuition of the mesenteric blood flow occurs. This allows blood to be shunted from nonvital to life-preserving tissues with the final result of an increase in mesenteric arterial vascular resistance and intestinal ischemia. After an acute hypoxic-ischemic injury, there are various reperfusion-associated events that can result in further damage to the intestinal mucosa mainly caused by free oxygen radicals[24], especially superoxide produced in postischemic tissues mediated by the xanthine oxidase reaction. Increased release of oxygen radicals from brain tissue of fetal lambs during partial occlusion of the umbilical circulation is explained by this xanthine oxidase reaction. A similar observation could be made in term infants with a nuchal umbilical cord[25].

The platelet activating factor (PAF) rapidly activates intestinal xanthine oxidase through proteolytic xanthine dehydrogenase-xanthine

oxidase conversion, predominantly in the ileal epithelium. This effect is mediated by neutrophils. Xanthine oxidase activation promotes PAF induced polymorphonuclear leucocyte sequestration into the intestine[26].

Inhibition by a monoclonal antibody (IB4) against neutrophils and by allopurinol treatment was paralleled by improvement of biochemical and morphological parameters. The radicals detected during reperfusion could therefore be divided into one component arising directly from the neutrophils and one due to the xanthine oxidase reaction. Neutrophils have to be recognized as a major source of radical production during reperfusion after ischemia[27].

The problem of ischemia and reperfusion is paramount and involves almost every vascular system. Because of its relevance in newborn intensive care ischemic acute renal failure should be mentioned. With oxygen radical scavenger pretreatment in experimental hypoxic acute renal failure of the rat this deterioration could be avoided.

Neutrophil cell invasion and oxidative burst

To any site of tissue injury, neutrophils are attracted and oxidative burst induced. Although superoxide ($O^{\cdot -}$) is the primary product of oxidative burst, its principal effect in microbicidal activity or host cell killing is via H_2O_2 and other ROS. The very aggressive hypochlorous acid (HOCl) is the most powerful oxidant produced by neutrophils and monocytes. It is generated from Cl- and H_2O_2 under the control of myeloperoxidase after cell stimulation by cytokines. Amines react to give chloramines, which have more limited oxidizing properties. Especially, HOCl reacts with taurine to form tauromonochloraminic acid, which is a longlived and relative selective bactericidal substance with a decreased general cellular toxicity.

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