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Total oxidant/antioxidant status in jaundiced newborns before and after phototherapy

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Abstract

Objective: To assess the effect of phototherapy on serum oxidant and antioxidant status in hyperbilirubinemic full-term newborns.

Method: Thirty-four full-term infants from 3 to 10 days of age exposed to phototherapy were studied. The serum antioxidant status was assessed by measuring the total antioxidant capacity (TAC) and individual antioxidant components: vitamin C, uric acid, albumin, thiol contents and total bilirubin. The oxidant status was assessed by determining the total oxidant status (TOS), oxidative stress index (OSI) and individual oxidant components: malondialdehyde (MDA), and lipid hydroperoxide levels.

Results: Vitamin C, uric acid, total bilirubin and MDA concentration were significantly lower, whereas serum TOS, lipid hydroperoxide and OSI levels were significantly higher after phototherapy ($p < 0.05$). There were significant positive correlations between serum total bilirubin and MDA ($r = 0.434$, $p = 0.001$).

Conclusions: Although the MDA level was reduced after phototherapy, phototherapy has a negative impact on numerous parts of the oxidant/antioxidant defense system in jaundiced full-term newborns, exposing them to potential oxidative stress.

J Pediatr (Rio J). 2007;83(4):319-322: Antioxidants, infant, malondialdehyde, oxidative stress, phototherapy.

Introduction

Phototherapy is the most widely used form of therapy for unconjugated hyperbilirubinemia.[1,2] Its noninvasive nature, easy availability, low cost and occurrence of few side effects have initially almost led to the assumption that it is innocuous.[3] The possibility that this may not be the case has been raised in several recent publications, which have shown that phototherapy is a photodynamic stress and can induce lipid peroxidation. Increasing appreciation of the causative role of oxidative injury and lipid peroxidation in the development of many severe diseases of the newborn has lent tremendous importance to lipid peroxidation and its possible causes.[4]

Free radicals and related metabolites have attracted a great deal of attention in recent years.[5] They are mainly derived from oxygen and are generated in the body by various endogenous systems, exposure to different physicochemical conditions or pathophysiological states. Free radicals can adversely alter lipids, proteins and DNA, and have been implicated in pulmonary oxygen injury, intraventricular hemorrhage, retinopathy of prematurity, ischemia/reperfusion injury manifested as necrotizing enterocolitis, postasphyxial central nervous system injury, and acute tubular necrosis.[6,7] Reactions of bilirubin involving free radicals or toxic oxygen reduction products have been well documented: unconjugated bilirubin scavenges singlet

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oxygen with high efficiency, reacts with superoxide anions and peroxyl radicals, and serves as a reducing substrate for peroxidases in the presence of hydrogen peroxide or organic hydroperoxides.[8,9]

We hypothesized that an important factor in the mechanism of oxidative stress in hyperbilirubinemic full-term infants submitted to phototherapy on the first days of life would be increased oxidative stress in relation to antioxidants. This imbalance would be affected by exacerbated oxidative stress, diminished antioxidants, or a combination of both. The aim of this study was to test the validity of this hypothesis by determining the relative roles of oxidative stress and diminished total antioxidant activity.

Methods

Fifty-seven full-term newborn infants from 3 to 10-days of age who were delivered vaginally and admitted to Sanliurfa Children's Hospital because of clinically significant indirect hyperbilirubinemia comprised the subjects of this study. All the infants were being breastfed and had no etiological factor for hyperbilirubinemia. Infants with severe congenital malformation, maternal diabetes, birth asphyxia, sepsis or hemolytic-type hyperbilirubinemia due to blood group (Rh or ABO) incompatibility; those that had received intensive phototherapy; those in which the total serum bilirubin level rose by more than 5 mg/dL per day or was higher than 20 mg/dL within the first 24 hours after birth; and those with signs and symptoms suggestive of serious illness were excluded from the study. Clinically significant indirect hyperbilirubinemia was defined as being present in infants with a serum total bilirubin concentration of more than 13 mg/dL.[10] The naked newborn, except for those who wore diapers and eye patches, was placed inside an incubator with a phototherapy system consisting of six white fluorescent tubes (Philips TL 20W/54) 40 cm above. The light energy of the phototherapy unit, measured using a standard photometer (Light Meter VF, Minolta, Japan), was 12-16 $\mu W/cm^2/nm$. All babies were exposed to continuous phototherapy for 48 h, except while being fed, cleaned and sampled. This period of time was chosen so as to allow sampling simultaneously with routine bilirubin tests, thus avoiding blood sampling solely for the purpose of the study. This study was approved by the local Research Ethics Committee. All parents signed a consent form for the participation of newborns in the study.

Analytical methods

Blood samples were taken from a peripheral vein to determine total bilirubin, direct bilirubin, and antioxidant and oxidant concentrations prior to phototherapy. Second blood samples were taken from 49 infants after 48 h. Blood samples were centrifuged at 1500 x g for 10 minutes within 20 minutes of collection. Serum samples were stored at -80°C and analyzed within 2 months.

Total antioxidant capacity (TAC) levels were measured by Erel's TAC method,[11,12] which is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants. The results were expressed in mmol Trolox equiv./L. Serum thiol (total – SH group) content was measured by using dithionitrobenzoic acid (DTNB).[13] Vitamin C concentration was measured using the FRASC method.[14] Uric acid, albumin and total bilirubin, which are individual serum antioxidants, were measured using commercial kits (Abbott). Total oxidant status (TOS) serum concentrations were measured using Erel's TOS method,[15] which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange. The results were expressed in μmol H_2O_2/L. Erel's TAC and TOS methods are colorimetric and automated and the precision of this assay is excellent – lower than 3%.[13,16] Serum malondialdehyde (MDA) levels were measured using a fluorimetric method.[17,18] Lipid hydroperoxide serum concentrations were also measured using the automated ferric-xylenol orange method.[19] The TOS to TAC ratio was regarded as the oxidative stress index (OSI).[20,21] To perform the calculation, the result unit of TAC, mmol Trolox equivalent/L, was changed to μmol Trolox equivalent/L, and the OSI value was calculated as follows: OSI = [(TOS, μmol/L)/(TAC, μmol Trolox equivalent/L) /100].

Statistical analysis

The statistical analysis of the data was carried out with the Statistical Package for the Social Sciences, version 11.0, for Windows (SPSS, Inc). The Student's *t* test for paired samples was used to compare the blood samples before and after phototherapy, assuming a 95% confidence interval. Bivariate associations between continuous variables were assessed by Pearson's correlation test. P-values less than 0.05 were considered to be statistically significant.

Results

The infants' mean age was 6±3 days, their mean length was 50±3.8 cm, their mean body weight was 3.1±1.4 kg, their mean head circumference ranged from 36.2±1.9 cm, and 29 of them were male, whereas 28 were female. Serum antioxidant/oxidant parameters before and after phototherapy are shown in Table 1. Serum TAC, thiol content and albumin levels were not altered after phototherapy. Serum vitamin C, uric acid, total bilirubin and MDA concentrations were significantly lower after phototherapy than before it ($p < 0.05$). Conversely, serum TOS, lipid hydroperoxide and OSI levels were significantly higher after phototherapy than

Table 1 - Comparison of oxidant and antioxidant serum parameters before and after phototherapy in jaundiced newborns. Data are given as mean ± SD

	Before phototherapy (n = 57)	After phototherapy (n = 49)	p*
TAC (mmol Trolox equiv./L)	1.54±0.31	1.48±0.13	0.281
Total – SH group (mmol/L)	0.42±0.01	0.43±0.01	0.165
Vitamin C (mg/dL)	2.1±1.3	1.3±0.6	0.029
Uric acid (mg/dL)	5.0±2.7	3.7±1.4	0.027
Albumin (mg/dL)	3.8±0.5	3.9±0.5	0.580
Total bilirubin (µmol/L)	17.1±2.5	13.8±2.3	< 0.001
TOS (µmol H_2O_2 equiv./L)	11.34±5.9	16.34±7.4	0.002
MDA (µmol/L)	2.46±0.36	1.98±0.33	< 0.001
Lipid hydroperoxide (µmol H_2O_2/L)	6.11±2	7.37±2.8	0.025
OSI (Arbitrary unit)	0.07±0.03	0.11±0.05	0.002

MDA = malondialdehyde; OSI = oxidative stress index; TAC = total antioxidant capacity; TOS = total oxidant status.
* Paired-samples *t* test.

before it ($p < 0.05$). There were significantly positive correlations between serum total bilirubin and MDA ($r = 0.434$, $p = 0.001$). Furthermore, there was no correlation between total bilirubin and other parameters.

Discussion

In the present study, vitamin C levels and uric acid, which are well known antioxidants, were significantly lower after phototherapy than before it; in contrast, TOS, lipid hydroperoxide and OSI levels were significantly higher after phototherapy than before it. Interestingly, MDA, which is known as an end product of lipid peroxidation and as an oxidant marker, was decreased after phototherapy. There was also a significant positive correlation between MDA and total bilirubin. All the published studies are related to the oxidative effects of phototherapy, especially lipid peroxide (which is a TBARs), and antioxidant enzyme activities, but not to serum nonenzymatic total antioxidant capacity.[22-27] This is the first report showing an association between these serum oxidant/antioxidant parameters in phototherapy-treated full-term nonhemolytic hyperbilirubinemic infants.

In a healthy human being, the formation and inactivation of reactive oxygen species are balanced at a level at which the compounds can play their physiological role without any toxic effects. This balance can be unstable in the neonatal period following rapid changes in tissue oxygen concentration, immature antioxidant mechanism and considerable neonatal developmental changes in antioxidants. This deterioration is especially evident in the presence of oxidative stress such as phototherapy.[4]

Although phototherapy is now widely used for the treatment of neonatal hyperbilirubinemia, there are concerns regarding the possibility of photodynamic tissue damage.[28] The exposure of infants to phototherapy in the presence of a sensitizer (bilirubin) resulted in oxidative injury to the red cell membrane manifested by a significant increase in concentrations of products of lipid peroxidation in the membrane and in hemolysis.[29] Another study reported that phototherapy in the presence of bilirubin induced a dramatic decrease in ATPase activity and an increased susceptibility to lipid peroxidation in neonatal erythrocytes.[4] Our study showed that phototherapy remarkably increased serum lipid hydroperoxides and TOS in jaundiced newborns.

It was shown that plasma MDA concentrations in neonates with nonhemolytic jaundice were significantly higher than those in healthy infants.[22,23] Ozture et al. reported that MDA concentrations were significantly lower after phototherapy than before it, and no significant correlation was found between plasma MDA and plasma bilirubin concentrations before and after phototherapy.[23] Yigit et al. reported that there was no significant correlation between MDA and bilirubin levels.[22] They also reported that both bilirubin and MDA were increased in jaundiced infants, and both parameters were reduced after phototherapy, but they did not find a significant correlation between the opposite oxidant/antioxidants parameters. The results of our study confirmed those findings, but we also investigated serum TOS, lipid hydroperoxide and OSI levels. In our study, serum MDA concentrations significantly decreased after phototherapy, and a significant correlation was found between plasma MDA and bilirubin concentrations before and after phototherapy ($r = 0.434$, $p = 0.001$). These results suggest that oxidative stress was not caused by phototherapy. However, TOS, lipid hydroperoxide and OSI levels were significantly increased after phototherapy. The apparent cause of these conflicting data is the fact that MDA measurement is not a specific method for lipid peroxidation, and that it is positively affected by bilirubin and some aldehyde structures.[15] MDA is an end product of the lipid peroxidation process, and lipid hydroperoxide, which is our lipid peroxidation parameter, is an early marker of the oxidation chain of lipids, and in the latter method, there is no interference with other structures.

Previous studies examined the effect of phototherapy on erythrocyte antioxidant enzyme activity (glutathione peroxidase, superoxide dismutase, etc.) in jaundiced infants. We investigated serum nonenzymatic total antioxidant capacity and individual antioxidants. Bohles et al. reported a significant decrease in serum uric acid during phototherapy.[30] The decrease in serum uric acid concentration is discussed as an effect of direct photodecomposition on the one hand, and as an inhibitory effect of riboflavin deficiency on uric acid formation on the other hand. We found that TAC levels were not significantly altered by phototherapy, but

vitamin C and uric acid concentrations were significantly reduced. However, TAC levels were not reduced, and the oxidant/antioxidant balance shifted significantly to the oxidant side, because other indicators of oxidative status, i.e. lipid hydroperoxides, TOS and OSI levels, were significantly increased in jaundiced newborns exposed to phototherapy.

In conclusion, phototherapy has a negative impact on numerous parts of the oxidant/antioxidant defense system in newborn hyperbilirubinemic infants, exposing them to potent oxidative stress.

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