Detection of secretory immunoglobulin A (SIgA) in saliva of ventilated and non-ventilated preterm neonates

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Abstract

The very young preterm neonate has multiple immune deficiencies which may increase his or her vulnerability to infection. Secretory Immunoglobulin A (SIgA) plays an important role in the protection of epithelial surfaces exposed to the external environment; nevertheless controversy exists with regards to the ontogeny of SIgA in newborns and especially the preterm neonate. The objective was to investigate if SIgA could be detected in the saliva of very/extremely low birthweight neonates (V/ELBW). A total of 707 samples which were collected twice daily (morning and afternoon) for three consecutive days were obtained from sixty-eight preterm neonates (mean gestational age 28 weeks; conceptional age ranged from 25–35 weeks). A repeated measures design was used. Total concentration of SIgA was determined from unstimulated saliva by an Enzyme Linked Immunosorbant Assay technique. Results indicated that SIgA was detectable in the early postnatal period in the saliva of both ventilated preterms who were receiving intravenous total parenteral nutrition (TPN) and non-ventilated preterms. A 3-way repeated measures Analysis of Variance (ANOVA) showed no significant effect from ‘before’ and ‘after’ samples during a period of spontaneous activity, time and day of sampling. A significant effect of mode of nutrition was found; neonates who were receiving expressed breast milk had significantly higher concentrations of SIgA than those infants receiving TPN (df=3, F=14.27, p<0.0001). These results have implications for the care of the preterm neonate in intensive care.
Introduction

Multiple immune deficiencies in infants born prior to twenty-eight weeks gestational age have been shown to predispose them to increased vulnerability of bacterial invasion (Yodfat & Silvian 1977). Secretory Immunoglobulin A (SIgA) plays an important role in the protection of epithelial surfaces exposed to the external environment, in particular those of the upper respiratory tract (Brandtzaeg et al. 1990), and is often utilized as an indirect marker of mucosal immunity (Burgio et al. 1980). SIgA can be detected in tears, saliva, respiratory and gastrointestinal tract mucosal secretions.

Controversy exists as to whether SIgA is detectable in the saliva of the newborn. Some studies concluded that there was no SIgA in the saliva of neonates at the time of birth (Smith & Taubman 1993; Cripps, Gleeson & Clancy 1991; Iwase, Moro & Mestecky 1987; Mellerand, Carlsson & Hanson 1986). These studies did however detect rapidly rising concentrations in the early months after birth. In contrast other research suggested that some of the SIgA antibodies detected in neonatal saliva were possibly fetal in origin (Thrane, Rognum & Brandtzaeg 1990). Immunoflorescence studies (Cripps, Gleeson & Clancy 1991), using fetal tissue, also indicated the potential for an immune response. Indeed small but detectable concentrations of IgA in the salivary glands of fetuses and neonates, including premature neonates, have been noted (Sennhauser, Balloch, Shelton, Doyle, Yu & Robertson 1990; Fitzsimmons, Evans, Pearce, Sheridan, Wentzen & Cole 1994). One study isolated SIgA and its subclasses (IgA1 and IgA2) as early as three days after full-term birth (Friedman, Entin, Zedeka & Dagan 1993) and small concentrations of serum IgA have also been detected in the cord blood of very young infants within the first week after birth (Kugler, Hess & Haake 1992).

Extreme variations in individual data have been identified (Smith & Taubman 1993; Cripps, Gleeson & Clancy 1991; Whitelaw & Parkin 1988) which were attributed to environmental mitogenic influences; due to individual variation the definition of normal ranges remains difficult. This study was designed to test the hypothesis that SIgA could be detected in the saliva of ventilated preterm neonates who were receiving intravenous total parenteral nutrition (TPN) during their first days of postnatal life. Some authors (Atkinson et al. 1990; Milla & Bissett 1988) have suggested that SIgA concentrations were influenced by the mode of nutrition; SIgA detected in maternal breast milk is thought to play a role in the protection of the neonate from respiratory and gastrointestinal tract infections (Smith & Taubman 1993); consequently the authors also report here on results of concentrations of SIgA in non-ventilated preterm neonates who were being fed with breast milk and/or formula.

Method

Design

A repeated measure design was used by collecting baseline data three minutes ‘before’ and three minutes ‘after’ a period of spontaneous activity.

Participants

Seventy neonates (33 male, 37 female) were recruited from four neonatal intensive care units in Wolverhampton, Shrewsbury and Gloucester where the Ethics Committee, senior consultant and parental consent had been obtained. Gestational age (GA) ranged from 24 to 34 weeks (Mean = 28; SD = 2.3), and birthweight (BW) from 0.65 to 2.44 kg (Mean = 1.16 kg; SD = 0.38).

Twenty-nine neonates were delivered by caesarean section and the remainder by spontaneous vaginal delivery. Thirty-eight required mechanical ventilation (IPPV), five needed continuous positive airways pressure ventilation (CPAP), and the remaining r (n = 25) required no ventilatory assistance apart from supplemental oxygen via face mask as necessary. Twenty-one of the infants were receiving morphine as a sedative. There were twelve cases of confirmed sepsis and thirty-four cases of presumed sepsis. There were four cases of necrotizing enterocolitis and one of these infants died after the study period.

Thirty-four (50%) ventilated preterm neonates were receiving intravenous total parental nutrition (TPN); a further thirty-four (50%) non-ventilated preterm neonates were fed by formula (FORM; n=15), expressed breast milk (EBM; n=.13) or by EBM and FORM (MIX; n=6).

Mean postnatal age for ventilated neonates who received total parental nutrition was 3 days (SD +/−1.5), with a mode of one postnatal day relating to the day of the study when the first sample was taken. Formula fed neonates had mean postnatal age of 8 days (SD +/−5.4) mode of 3 postnatal days when the first sample was taken; neonates receiving expressed breast milk had a mean postnatal age of 8 days (SD +/−5.4) when the first sample was obtained with a mode of two postnatal days.

Procedure

Unstimulated saliva samples were obtained from just inside the buccal cavity of the neonates and were obtained when the neonate had been lying alone for forty-five minutes to one hour. This time was dependent on the medical stability of the infants. The sam-
samples were obtained using a small sterile flexible plastic filament attached to a sterile 5 ml syringe, and were transferred to a 1 ml ependorf, returned to the University on ice where they were stored at −20°C until analyzed.

Samples were obtained when the neonate was lying alone with no intervention taking place (spontaneous activity). Where medical stability allowed within the first two weeks after birth, this procedure took place once in the morning and once in the afternoon on three consecutive days. The mean postnatal age of the neonates at the commencement of the study has been referred to in the section on participant details. In total 707 samples were obtained from sixty-eight of the neonates when it should have been 816; twenty-one samples (5%) did not have sufficient saliva for Secretory IgA to be detected; 44 sessions (88 samples) could not be carried out for various reasons, including medical instability of the neonate.

An enzyme-linked immunosorbant assay, ELISA, method was used to ascertain SlgA concentrations using Sigma chemicals (Poole, Dorset, U.K.). All standards and samples were run in duplicate. Each well on the ELISA plate was coated with 100 ml of an optimum concentration of rabbit anti-human IgA (a chain specific), diluted 1:800 in Coating buffer 0.16% Na2CO3 and 0.29% NaHCO3 pH 9.6, and incubated for one hour at 37°C. Following incubation the wells were washed five times in washing buffer (0.8% NaCl; 0.02% KCl; 0.02% KH2PO4; 0.29% Na2HPO4; and 0.1% Tween 80 pH 7.2).

A blocking solution (100µl of casin 2% in Phosphate Buffer Solution, [PBS, pH 7.6]) was then introduced into each well in order to minimize non-specific binding, and again incubated for one hour at 37°C and washed as previously. 100µl of the saliva samples (diluted1:25) or 100µl of purified secretory human IgA, for the generation of the standard curve (range 0–100µg/ml), was then added to the wells. After a further incubation and washing step 100µl of goat anti-human IgA (a chain specific) peroxidase conjugate (diluted 1:1000 in PBS) was added to the wells and a further hour incubation was followed by washing as before. 100µl of ABTS substrate reagent was then added and the color was allowed to develop for an average of five minutes before the plate was read in a Labsystems Multiskan MS (type 352) plate-reader at absorbance wavelength of 414nm. Secretory IgA concentrations were estimated from the standard absolute values, obtained from each plate using semilogarithmic linear regression analysis. The concentrations obtained were then multiplied by the dilution factor of 1:25 to give SlgA concentration in µg/ml. Any sample whose value fell below that of the control well for no IgA was discarded.

Results

Statistical analyses were conducted on 707 samples using the SuperAnova software package. A wide intra- and inter-individual range of SlgA concentrations were observed (2.1 to 7231.9 µg/ml). Table 1 illustrates the values obtained from ‘before’ and ‘after’ sampling during the period of spontaneous activity (SA).

<table>
<thead>
<tr>
<th>'Before’/‘After’</th>
<th>Mean SlgA (µg/ml)</th>
<th>Standard Error (Standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Before’</td>
<td>217.97</td>
<td>47.51</td>
</tr>
<tr>
<td>‘After’</td>
<td>263.22</td>
<td>65.71</td>
</tr>
</tbody>
</table>

A 3-way repeated measures analysis of variance (ANOVA) 2 X (Before/After) X 2 (a.m./p.m) X 3 (Days 1, 2, 3) was conducted. There was neither significant effect between the ‘before’ and ‘after’ samples (df=1, F=0.2, p<0.7), nor were interactions indicated for time of day ((df=1, F=0.9, p<0.4) or between the three days (df=2, F=0.2, p<0.8).

Due to the presence of SlgA in breast milk it was of interest to observe if those infants so fed had differing concentrations of SlgA than infants who received formula, or a mixture of the two, or intravenous total parental nutrition (TPN). Details for these three variables are illustrated in Figure 1.

Analysis of variance indicated a significant effect of mode of nutrition (df=3, F=14.27, p<0.0001). Post hoc analysis using Tukey Kramer found those infants who had received expressed breast milk feeds had a higher SlgA concentration than all other modes of nutrition.

Discussion

This study tested the hypothesis that SlgA could be detected in the first days of postnatal life of ventilated preterm neonates who were receiving intravenous total parental nutrition (TPN). Results supported the hypothesis and are encouraging. Hitherto there has not been a study reporting concentrations of SlgA on this type of population, this having no implications for the care of preterm neonates in intensive care. One may speculate that if SlgA is...
present at such early age, attention should be given to investigating how these concentrations could be increased. An enhanced immune system would doubtless assist such vulnerable neonates in fighting infection which is so common in ventilated preterms.

The mean value of concentration of SIgA detected in these neonates, albeit significantly lower than the concentrations detected in the sample of neonates fed with breast milk, are still high. The mean concentration of 107.45 µg/ml of this study is similar to that found in adults by Tappuni & Challacombe, i.e., 227 for SIgA sub-class 1 and 123 to SIgA sub-class 2, and greater than the values found, for example, by Friedman et al. 1996, the latter authors, for a smaller (n = 14) sample of full-term neonates, reported mean concentrations of SIgA1 of 6.4 µg/ml (SD+/-3.6), ranging from 0.0 –>39 µg/ml, while the mean concentration of SIgA2 was 2.6 µg/ml (SD+/3.0).

It may well be that SIgA is detectable in the preterm infant early in the neonatal period due to high exposure to antigenic stimulation (colonization by commensal bacteria, handling by many individuals, endotracheal suctioning) resulting in a rapid postnatal response.

The literature quoted here reported a wide variation in the concentrations of Secretory IgA (SIgA). These values ranged from barely detectable, as in this study, to relatively high concentrations. Nevertheless individual infant samples varied minimally across three consecutive days; thus differences cannot be attributed to interassay variation. Such large interindividual variations have also been reported by other authors, e.g. Smith & Taubman 1993; Cripps, Glesson & Clancy 1991. The differences in concentrations found between studies can also be attributed to the use of different methodologies and assay-sensitivity as pointed out by Friedman, Entin, Zedeka & Dagan (1996).

The very and extremely low birthweight may not receive oral nutrition in the first few days of life after birth due primarily to decreased gut motility (Milla and Bissett 1988) and severity of neonatal illness (especially respiratory). Secretory IgA is present in breast milk and may confer some increased immunity to the neonate receiving such nutrition (Atkinson et al. 1990; Milla & Bissett 1988).

The present study confirms increased salivary SIgA concentrations for infants who received expressed breast milk feeds. Nevertheless the higher concentrations of SIgA may also partially be due to the fact that the infants receiving breast milk were generally older than the neonates who relied on TPN for their source of nutrition.

There may be some concern that this sample is not homogeneous and is composed of two groups, i.e. (1) neonates who required intravenous total parental nutrition (TPN) and were ventilated and (2) neonates who received enteral nutrition and were non-ventilated. Accordingly the authors acknowledge, but emphasize that SIgA was detected in the saliva of 34 ventilated preterm neonates with no contact with breast milk and as early as 48 hours after birth. This study was designed to be carried out for only three consecutive days; further investigations should be carried out for at least 12 weeks of postnatal age.

In conclusion, Secretory IgA has been detected in the saliva of ventilated preterm neonates as early as the second day after birth. This study has, for the first time, included those infants who require mechanical ventilation and intravenous feeding and are therefore often referred to as medically unstable. Wide variations in Secretory IgA reflected interindividual differences; no differences in SIgA were detected over the time period of the study.

As Friedman et al. (1996) pointed out, it is now accepted that mucosal IgA and serum IgA belong to separate compartments of the immune system, although the relationship between these two is not yet clearly understood. Finding ways to enhance the immune system of the fragile ventilated preterm neonate prone to infections, who cannot yet be breast fed, is the aim of our future studies.

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