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Secretory IgA in the Saliva of Ventilated Preterm Neonates; the Mediating Role of Light and Systematic Stroking

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Abstract: Secretory Immunoglobulin A (SIgA) plays an important role in the protection of epithelial surfaces exposed to the external environment. The very young preterm neonate has multiple immune deficiencies which may increase his or her vulnerability to infection; previous studies have shown that SIgA can be detected in the saliva of preterm neonates during their first week of postnatal life. This study investigated the effects of a gentle and light systematic stroking therapy (TAC-TIC therapy) on the secretory immune response of 35 ventilated, very and extremely low birthweight (V/ELBW) preterm neonates (17 male, 18 female), who were receiving intravenous total parenteral nutrition (TPN). All neonates received the therapy for three consecutive days using a repeated measures counterbalanced design. Saliva was obtained both before and after TAC-TIC (135 sessions) and a control time period of spontaneous activity (129 sessions). Repeated measures analysis of variance indicated a significant difference by condition ($df = 1, F = 4.87, p < 0.05$) between before and after variables; further analysis indicated that significant differences were detected from before to after the therapy only ($df = 1, F = 5.03, p < 0.05$). Following this tactile stimulation programme, possible implications include facilitation of the development of the secretory immune system. The self-regulatory concepts of a psychoneuroimmunological model of tactile stimulation provide a satisfactory explanation of the results.

Zusammenfassung: Sekretorisches Immunoglobulin A (SIgA) spielt eine wichtige Rolle beim Schutz der der äußeren Umwelt ausgesetzten epithelialen Oberflächen. Die sehr

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Preliminary results of this study have been presented at the ICIS Conferences held at Rhode Island, 1996 and Atlanta, 1998; a Summary of results has been quoted in Adamson-Macedo (1997, 1998).

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jungen Frühgeborenen haben vielfältige Immunschwächen, die seine oder ihre Infektionsanfälligkeit steigern können. Frühere Studien haben gezeigt, daß SIgA im Speichel von Frühgeborenen während der ersten Wochen des postnatalen Lebens nachgewiesen werden kann. Diese Studie untersucht die Wirkungen einer sanften, leichten aber systematischen Streichel-Therapie (TAC-TIC-Therapie) auf die sekretorische Immunantwort bei 35 beatmeten, unreifen Frühgeborenen (17 männlichen, 18 weiblichen) mit extrem niedrigen Geburtsgewicht (V/ELBW), die eine intravenöse und vollständig parenterale Ernährung (TPN) erhielten. Alle Neugeborenen erhielten die Streichel-Therapie über drei aufeinander folgende Tage, wobei ein durch wiederholte Messungen balanciertes Design gewählt wurde. Der Speichel wurde vor und nach der Streichel-Therapie (TAC-TIC, insgesamt 135 Sitzungen) gewonnen, und als Kontrollzeit diente eine Phase spontaner Aktivität (129 Sitzungen). Die Methode der wiederholten Meßanalysen der Varianz ergab eine signifikante Differenz ($df = 1, F = 4.87, p < 0.05$) der Differenz zwischen dem Zustand vor und nach den Variablen. Die weitere Auswertung ergab, daß diese Differenz nur vor und nach der Therapie meßbar waren ($df = 1, F = 5.03, p < 0.05$). Aus diesen Ergebnissen ergibt sich eine mögliche Förderung der Entwicklung des sekretorischen Immunsystems. Die Annahme eines selbstregulatorischen Prinzips des psychoneuroimmunologischen Modells einer taktilen Stimulation ergibt eine ausreichende Erklärung für die Ergebnisse.

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Introduction

As pointed out by Goldberg and Divitto (1983), the premature infant has to cope with a very stressful environment, is prone to respiratory complications, and is more vulnerable to infections than healthy full-term counterparts. The mediation of antibody activity is the primary function immunoglobulins (Sha, Vincent and Compans 1999), and the vast majority of human pathogens initiate their infections at the mucosal surfaces of the respiratory gastrointestinal or urogenital tracts. Secretory Immunoglobulin A (SIgA) primarily appears in tears, saliva, and in mucosal secretions of the respiratory and gastrointestinal tracts, and 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); SIgA is often utilised as an indirect marker of mucosal immunity (Burgio et al. 1980).

This paper shows that secretory immunoglobulin A (SIgA) is found in the saliva of ventilated very/extremely low birthweight (V/ELBW) preterm neonates, and further indicates that IgA responses in such secretions are elicited by gentle and *light* systematic stroking (TAC-TIC therapy)[1].

Although the time at which SIgA is detected in the newborn remains controversial, SIgA has been detected in the tracheobronchial aspirate of premature infants at birth (Sennhauser et al. 1990). Some studies concluded that there was no SIgA in the saliva of neonates at the time of birth (Smith and Taubman 1993; Cripps, Gleeson and Clancy 1991; Iwase, Moro and Mestecky 1987; Mellander, Carlsson and Hanson 1986), whilst others suggested that some of the SIgA antibodies detected in neonatal saliva were possibly foetal in origin (e.g., Thrane, Rognum and Brandtzaeg 1990; Friedman et al. 1993). Small concentrations of

[1] Touching And Caressing; Tender In Caring.

serum IgA have also been detected in the cord blood of very young infants within the first week after birth (Kugler, Hess and Haake 1992).

High interindividual variations in SIgA concentrations are however often reported in adult populations (Kugler et al. 1992) and in those of children and infants (Burgio et al. 1980). Hayes, Adamson-Macedo, Perera and Anderson (1999) have shown that SIgA was detected in ventilated neonates soon after birth.

Increased morbidity and mortality in low birth weight, premature infants has also been attributed to low levels of immunoglobulins (Malik, Yadav and Sehgal 1980). Moreover, in coping with "long-term stress", vulnerability to infection of the ventilated preterm neonate may well increase; the secretory immunological changes in relation to stress and stress relief and the development of the secretory immune system in infants has hitherto received little attention, although the relationships between the mind, nervous system and endocrine/immune systems of the preterm neonate is addressed by Neonatal Developmental Psychoneuroimmunology (Adamson-Macedo 1997). The basis of this sub-discipline is Gottlieb's (1992) system view of development which considers epigenesis as probabilistic, and the individual as an 'emergent, coactional, hierarchical system'. The psychoneuroimmunological model of tactile stimulation asserts that an appropriate psychological intervention should be able to modulate the secretory immune system of the "at risk" ventilated 'coactive' preterm neonate.

A review by Kugler (1995) showed that relationships between either short or long-term stress and uppper respiratory tract infections (URTI) have recently received attention. Decreased concentrations of SIgA have been associated with periods of physical and psychological stress and negative mood states (Kugler, Hess and Haake 1992; Khansari, Murgo and Faith 1990; Jemmott et al. 1983).

Related work by Green and Green (1987) noted that SIgA secretion increased rapidly following a twenty-minute back massage applied to adults and following periods of relaxation, and Harbuz, Burns, Bunt and Hucklebridge (1999) indicated that listening to music produced an increase in SIgA levels.

None of the above contains any record of studies involving ventilated preterm neonates. Beneficial effects of different early sensory interventions on neurophysiological, psychological, behavioural and cognitive development of infants born preterm have been reported extensively for the past three decades (e.g., Korner et al. 1975; Rice 1978; Masi 1979; Adamson-Macedo 1985; Schanberg and Field 1986; Achenbach et al. 1990; Lester and Tronick 1990; Ludington-Hoe, Hadeed and Anderson 1991; de Roiste and Bushnell 1991, 1996; Adamson-Macedo et al. 1993; Acolet et al. 1993; Adamson-Macedo et al. 1994; Hayes and Adamson-Macedo 1998; Adamson-Macedo 1998). None of these were systematic studies of the role of early tactile stimulation in facilitating the development of the secretory immune system of the ventilated preterm neonate, and first such report was given by Hayes, Adamson-Macedo, and Perera in 1996. The present study has tested the hypothesis that gentle and light systematic stroking (TAC-TIC therapy) elicits SIgA responses in very/extremely low birthweight ventilated preterm neonates who are in receipt of intravenous total parental nutrition (TPN) during their first week of postnatal life.

Method

Thirty-five infants (17 male, 18 female) were recruited from four neonatal intensive care units in general hospitals in the Midlands, Shropshire and Gloucestershire; necessary parental and Ethics Committee approval were previously obtained for each hospital. Infant details are illustrated in Table 1. Entrance criteria were a birthweight of less than 1.5 kg, a gestational age of less than 32 weeks, and ventilation for a minimum of three days and less than one week postnatal age.

Table 1. Infant Details

	Mean value	Standard deviation	Standard error	Range
Gestational Age (weeks)	27	1.2	0.2	25–30
APGAR* score at 1 minute	5	2.4	0.4	1–9
APGAR* score at 5 minute	8	2.3	0.4	2–10
Birthweight (kg)	0.995	0.02	0.04	0.645–1.42

* APGAR scores are a medical definition of well-being at birth that incorporates colour and breathing; range 1–10, where 10 is the highest score.

Fourteen of the neonates were delivered by caesarean section and twenty-one by spontaneous vaginal deliveries. All infants required mechanical ventilation; mean oxygen requirement was 28% O_2. Twenty-three of the infants were receiving morphine as a sedative. All the infants were treated with prophylactic antibiotics, and there were eight incidences of confirmed sepsis, eighteen presumed sepsis, and nine infants who had no signs of sepsis. One infant was diagnosed as having necrotising enterocolitis (NEC) and subsequently died, and there were two cases of suspected NEC in this group during the period of study.

Design

An interrupted time series counterbalanced design was employed which allowed for the recording of data before and after the intervention or control condition (spontaneous activity).

Intervention and Control

A modified version of TAC-TIC therapy (version-3; Hayes, 1996) was used; this involved a programme of gentle and *light* skin-to-skin, systematic stroking following a cephalocaudal pattern (i.e., head to foot) which took between 3–4 minutes to complete. This method adopted three abdominal movements by placing the hand just above the infants torso; in this version there are no repetition of movements. TAC-TIC technique was taught by the originator of the therapy, and was uniformly applied to all the babies in the study. The control was spontaneous activity defined as a period of time when no intervention was taking place and the infant was lying alone.

Procedure

Unstimulated saliva samples were obtained from just inside the buccal cavity of the neonates, before and after TAC-TIC therapy (n = 135 sessions) or a near-equivalent time period of spontaneous activity (n = 129 sessions). The samples were obtained using a small sterile flexible plastic filament attached to a sterile 5 ml syringe, and were transferred to a 1ml ependorf and then returned to the University on ice where they were stored at 20 °C until analysed.

Samples were obtained when the neonate was lying alone with no intervention taking place (spontaneous activity). Within the first two weeks after birth, where medical stability allowed, this procedure took place once in the morning (between 10–11 a.m.) and once in the afternoon (between 1–2 p.m.) on three consecutive days. The mean postnatal age of the neonates at the commencement of the study is given in the section on participant details.

An enzyme-linked immunosorbant assay method (Sigma Chemicals, Poole, Dorset, U.K.) was used to ascertain SIgA concentrations. Intra-assay coefficient of variation was 11%. All standards and samples were run in duplicate. Each well on the ELISA plate was coated with 100 μl of an optimum concentration of anti-human IgA (Rabbit α chain specific), diluted 1:800 in Coating buffer 0.16% Na_2CO_3 and 0.29% $NaHCO_3$ 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% KH_2PO_4 0.29% Na_2HPO_4 and 0.1% Tween 80 pH 7.2) and blotted.

A blocking solution (100 μl of casin 2% in Phosphate Buffer Solution [PBS, pH 7.6]) was then introduced into each well in order to minimise non-specific binding, and again incubated for one hour at 37 °C, washed and blotted as previously. 100 μl of the saliva samples (diluted 1:25) or 100 μl of purified secretory human IgA, for the generation of the standard curve (range 0–100 μg/ml) was then added to the wells. After a further incubation and washing step 100 μl of anti-human IgA (Goat α chain specific) peroxidase conjugate (diluted 1:1000 in PBS) was added to the wells and a further hour of incubation was followed by washing and blotted as per protocol. 100 μl of ABTS substrate reagent was then added and the colour 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 414 nm.

Results

Of the thirty-five infants recruited, sufficient sample size of saliva for analysis were obtained from thirty two. 492 samples were obtained, 252 (126 before; 126 after) from the experimental condition and 240 (120 before; 120 after) from the control condition. Results were analysed using the mean of the duplicate samples for each infant. A wide range of SIgA concentrations were detected in the samples (3.1–5004.5 μg/ml); the volumes obtained were in a range from 50–200 μl. A repeated measures analysis of variance (MANOVA) for 2 X Condition (TAC-TIC / Control), 2 X Time of Day (AM/PM), 3 X Day of Study (1/2/3) and 2 X Before/After indicated no significant effect of time of day ($df = 1$, $F = 0.07$, $p < 0.8$) or day of the study ($df = 2$, $F = 0.29$, $p < 0.8$). A significant interaction was found

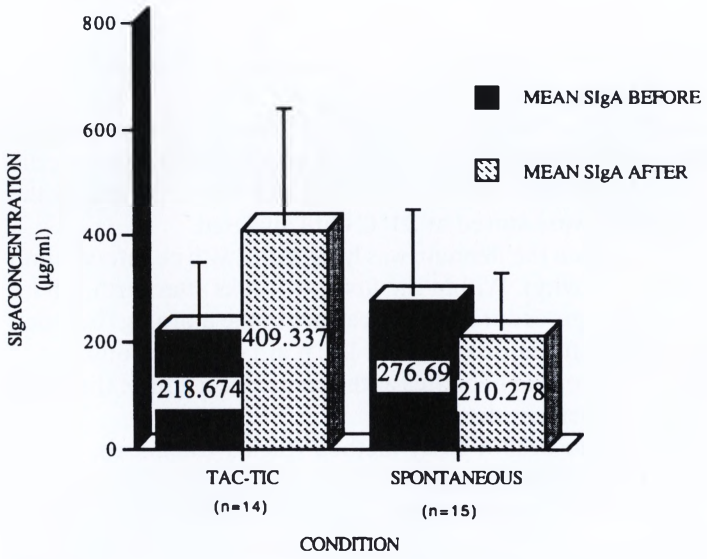

Fig. 1. Mean salivary SIgA concentration (µg/ml) before and after TAC-TIC and spontaneous activity

for conditions before and after samples ($df = 1$, $F = 4.87$, $p < 0.05$). Further analysis indicated that there were no significant differences from before to after the control condition ($df = 1$, $F = 3.72$, $p < 0.09$), and that SIgA concentrations significantly increased ($df = 1$, $F = 5.03$, $p < 0.05$) from before to after the tactile stimulation intervention. See Fig. 1 for further details.

In addition, ANOVA indicated no effect on SIgA concentration neither with or absence of sepsis ($df = 2$, $F = 0.77$, $P < 0.5$). Neither was there any effect of gender on SIgA concentration [Males: Mean 43.32 SE $\pm$ 4.2; Females: Mean 26.35, SE $\pm$ 3.90; ($df = 1$, $F = 0.92$, $p < 0.4$)].

Discussion

The results indicated that SIgA could be detected and recorded in very/extremely low birthweight ventilated, preterm neonates early in the postnatal period (i.e., during the first week of life). Variations in concentration could be attributed to inter-individual differences as discussed by Kugler, Hess and Haake (1992). None of the infants were receiving expressed breast milk, and consequently variations observed cannot be attributed to maternally transferred SIgA. The study may also support the concept that the secretory immune response is initiated almost immediately post partum.

Unless a possible discomfort or internal stressor such as pain was being, observation of any differences in SIgA concentration following a period of spontaneous activity, could not be expected, and conforms with results which show a decrease in SIgA; however, significant increases in SIgA were found following tactile stimulation, thus supporting the hypothesis that SIgA can elicit IgA responses in the secretions which represent a key factor in the prevention of infections. Moreover,

the results suggest that the therapy is enhancing the secretory immune system of the neonate. Anticipatory effects whereby the infants may have expected TAC-TIC to follow use of the syringe which collected the saliva, and be distressed by absence of the therapy, can be rejected as the methodology of the study ensured that the intervention and the control period were counterbalanced.

Overall, the study suggests that it is the gentle/*light*, systematic use of this particular tactile stimulation therapy which alleviates the distress of the neonates. It is possible that oxygen therapy may be an irritant to the delicate mucosal membranes of the premature infant, and this may affect the secretion of SIgA. However, the mean oxygen requirements of the infants was 28%, a figure which is not excessively high.

According to Gottlieb (1976, 1992) experiences or coactions between the various organic and psychological systems of the individual, as well as with his or her environment, may facilitate, maintain or induce development. It follows that the intervention utilised in this investigation could play a facilitative role in the development of the secretory immune system of these preterm neonates; such coactions of the sensory system and the immune system may help the preterm neonate's self-regulation mechanisms to develop. Albeit speculative, the results may have important implications to the ability to respond to infections of the upper respiratory tract, a complication to which this group of infants are particularly vulnerable.

The increase in secretion of SIgA following TAC-TIC therapy may decrease the incidence of reported infection, but has yet to be established. Explanation of the enhancement of the secretory response via non-pharmacological interventions has not hitherto been forthcoming and, in the absence of antigenic stimulation, is not fully understood; an explanation within a psychoneuroimmunological model is plausible, with the hypothesis that sensory afferent stimulation of the peripheral tissue during TAC-TIC therapy can be interpreted as being comforting (Hayes 1996) and may result in a decrease in stress hormones and associated increase in immune function.

It may confidently be concluded that gentle and *light* tactile stimulation in the form used in this study facilitates IgA salivary secretion responses in the very/extremely low birth weight preterm and ventilated neonate; further studies are investigating longer term implications for the quality of life and health status of these at-risk infants.

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