Summary

Background Preliminary studies suggested that pain experienced by infants in the neonatal period may have long-lasting effects on future infant behaviour. The objectives of this study were to find out whether neonatal circumcision altered pain response at 4-month or 6-month vaccination compared with the response in uncircumcised infants, and whether pretreatment of circumcision pain with lidocaine-prilocaine cream (Emla) affects the subsequent vaccination pain response.

Methods We used a prospective cohort design to study 87 infants. The infants formed three groups—uncircumcised infants, and infants who had been randomly assigned Emla or placebo in a previous clinical trial to assess the efficacy of Emla cream as pretreatment for pain in neonatal circumcision. Infants were videotaped during vaccination done at the primary care physician's clinic. Videotapes were scored without knowledge of circumcision or treatment status by a research assistant who had been trained to measure infant facial action, cry duration, and visual analogue scale pain scores.

Findings Birth characteristics and infant characteristics at the time of vaccination, including age and temperament scores, did not differ significantly among groups. Multivariate ANOVA revealed a significant group effect (p<0.001) in difference (vaccination minus baseline) values for percentage facial action, percentage cry time, and visual analogue scale pain scores. Univariate ANOVAs were significant for all outcome measures (p<0.05): infants circumcised with placebo had higher difference scores than uncircumcised infants for percentage facial action (136.9 vs 77.5%), percentage cry duration (53.8 vs 24.7%), and visual analogue scale pain scores (5.1 vs 3.1 cm). There was a significant linear trend on all outcome measures, showing increasing pain scores from uncircumcised infants, to those circumcised with Emla, to those circumcised with placebo.

Interpretation Circumcised infants showed a stronger pain response to subsequent routine vaccination than uncircumcised infants. Among the circumcised group, preoperative treatment with Emla attenuated the pain response to vaccination. We recommend treatment to prevent neonatal circumcision pain.

Introduction

Neonatal circumcision is a common surgical procedure in male infants. Despite evidence that circumcision causes intense pain and short-term alterations in infant feeding, sleeping, and crying behaviours, analgesia is rarely given. There is a common belief that the effects of circumcision pain are short-lived and clinically insignificant, and, therefore, that the benefits of analgesic treatment do not outweigh the risks of adverse effects from currently available therapies.

We looked at the foundations for the belief that the effects of circumcision pain are short-lived by examining infant behaviour several months after surgery. We analysed data from a clinical trial that studied the use of topical lidocaine-prilocaine 5% cream (Emla, Astra Pharma, Canada) during routine vaccination at 4 or 6 months. Male infants showed a greater pain response than female infants. This difference may be linked with neonatal circumcision in male infants. Male infants who had been circumcised also exhibited a greater pain response than those who had not been circumcised. This initial analysis raised concerns about the possible long-term effects of untreated pain in infants, especially those who have repeated experience of pain. However, we could not draw definite conclusions because of the post-hoc nature of the analysis and the small sample size. The objectives of our study were, therefore, to investigate prospectively whether neonatal circumcision affects infants' pain response to routine vaccination 4-6 months after surgery and whether vaccination response is affected by pretreatment of neonatal circumcision pain with Emla.

Methods

We carried out a prospective cohort study of 87 healthy, full-term, male, newborn infants who had, when aged 5 days or less, participated in a clinical trial that investigated the safety and efficacy of Emla cream for neonatal circumcision. The participants in this study included uncircumcised boys, who served as controls (n=32), and circumcised boys who had been randomly assigned treatment with Emla (n=29) or placebo (n=26) during circumcision. All parents who had allowed their infants to participate in the circumcision trial were asked to enrol their infants in this study and sign a consent form for their participation. We recruited uncircumcised infants from the same study by the same inclusion criteria as for the circumcised infants, the difference being that their parents had chosen not to have their infants circumcised. The protocol received approval from the Research Ethics Boards of the Hospital for Sick Children and Women's College Hospital.

The setting for this study was the clinic of the infant's primary care physician, where vaccination was done. Each infant's physician was contacted before the study commenced and informed about its purpose and procedures. One of the investigators telephoned all the parents 2-4 weeks before the anticipated date of the 4-month or 6-month vaccination to obtain details of the appointment date and time. We chose to study pain response during routine vaccination at 4 or 6 months to reduce the effects of fear and anticipation on infant pain response seen in older infants and children, and because vaccination pain responses do not vary greatly within this age range.

Parents were sent copies of the revised infant temperament questionnaire for infants aged 4-8 months to complete within...
the 2 weeks before the vaccination appointment. An investigator
met one or both parents and their infant at the primary
healthcare physician's clinic on the day of vaccination, and the
parents returned the completed questionnaire to the investigator at
that time.

The vaccination procedure was standardised across settings.
The infant was physically examined before the vaccination. If the
infant was unsettled by this examination, the parents were asked
to settle him. Immediately before the vaccination the infant was
placed supine on the examination table. A physician or nurse
then gave the infant an intramuscular injection of the vaccine
(0.5 mL, DPTE-Polio & Act-HIB, Connaught Laboratories
Ontario, Canada) in the left or right thigh. An investigator
recorded the infant's face for a minimum of 20 s with a video
camera (Panasonic, Ontario, Canada, model #PV-S770A-K),
before, during, and for up to 1 min after vaccination. Parents
were instructed not to hold the infant for the first 30 s after
the injection but were not discouraged from touching or speaking
to him during the procedure.

**Table 1: Flow of participating infants through trial**

<table>
<thead>
<tr>
<th>Category</th>
<th>Uncircumcised (n=32)</th>
<th>Circumcised with Emla (n=29)</th>
<th>Circumcised with placebo (n=26)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>4.1 (0.5)</td>
<td>4.2 (0.5)</td>
<td>3.9 (0.6)</td>
<td>0.22</td>
</tr>
<tr>
<td>Adaptability</td>
<td>2.3 (0.7)</td>
<td>2.0 (0.6)</td>
<td>2.1 (0.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Approachability</td>
<td>2.3 (0.7)</td>
<td>2.1 (0.7)</td>
<td>2.1 (0.6)</td>
<td>0.74</td>
</tr>
<tr>
<td>Distractionability</td>
<td>2.4 (0.7)</td>
<td>2.1 (0.6)</td>
<td>2.4 (0.6)</td>
<td>0.05</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 (0.8)</td>
<td>3.3 (0.7)</td>
<td>3.1 (0.7)</td>
<td>0.25</td>
</tr>
<tr>
<td>Mood</td>
<td>2.9 (0.8)</td>
<td>2.5 (0.6)</td>
<td>2.8 (0.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Persistence</td>
<td>3.3 (0.7)</td>
<td>3.2 (0.9)</td>
<td>3.5 (0.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>Rhythm</td>
<td>2.7 (0.6)</td>
<td>2.6 (0.9)</td>
<td>3.2 (0.9)</td>
<td>0.17</td>
</tr>
<tr>
<td>Threshold</td>
<td>3.7 (0.8)</td>
<td>3.8 (0.8)</td>
<td>3.5 (0.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>Overall temperament</td>
<td>2.1 (0.8)</td>
<td>2.3 (1.2)</td>
<td>2.3 (1.1)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

We used these three facial actions because they are particularly
sensitive for indicating pain.7

The three facial actions and cry duration were coded as
present or absent for each 1 s period of the 20 s before the
vaccination (baseline), and the first 20 s during and after
the vaccination. These data were then converted into percentages
of time that the infants exhibited the actions or cried (i.e.,
time—number of times action observed/20×100, where 20 was
the number of assessments made during the 20 s period).

An overall facial action pain score for the procedure
was calculated by adding together the facial action scores for the
three specific facial actions. The overall facial action pain score
 ranged from 0–100%. Percentage cry duration ranged from
0–100%. Visual analogue scale pain scores were rated with a
10 cm pain ruler.

The revised infant temperament questionnaire records the
relative frequency with which infants exhibit particular responses
to specified situations, such as feeding or bathing. Nine
temperament characteristics were derived from the questionnaire.
The revised infant temperament questionnaire has favourable
psychometric properties; internal consistency and test-retest
reliability coefficients are reported to be 0.83 and 0.86,
respectively.8 As well as being scored for the nine different
categories, infants were assigned an overall temperament rating of
easy (1), intermediate-low (2), slow to warm up (3),
intermediate-high (4), or difficult (5). The numerical scores were
used for ease of analysis.

On the day of vaccination, parents were asked questions about
their infant's last feeding and nap times, ingestion of paracetamol
for vaccination-fever prophylaxis, and previous painful
experiences. Information on birth characteristics and previous
vaccinations was obtained from the infant's medical records.
Socioeconomic status was scored by the Blishen scale9 based on
maternal occupation.

We based the calculation of necessary sample size on the
difference in pain scores between circumcised and uncircumcised
infants observed in our initial study of vaccination pain
responses,10 in which the mean visual analogue scale pain
score (unpaired) was 4.6 cm in the circumcised group and
2.7 cm in the uncircumcised group, and the SD was about
2.5 cm. Setting an α of 0.05 and β of 0.2, and to account for
possible drop-outs such as parents who refused to let their
children participate, those lost to follow-up, or those who could
not be included for reasons arising after initial contact, we
estimated that about 30 infants per group were needed.8

**Table 2: Demographic characteristics of infants at time of
vaccination**

<table>
<thead>
<tr>
<th>Category</th>
<th>Uncircumcised (n=32)</th>
<th>Circumcised with Emla (n=29)</th>
<th>Circumcised with placebo (n=26)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M (50%)</td>
<td>M (50%)</td>
<td>M (50%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Race</td>
<td>White (75%)</td>
<td>White (75%)</td>
<td>White (75%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Birthweight</td>
<td>3,100 (1,500)</td>
<td>3,100 (1,500)</td>
<td>3,000 (1,500)</td>
<td>0.86</td>
</tr>
<tr>
<td>Gestation age</td>
<td>37 (32)</td>
<td>37 (32)</td>
<td>37 (32)</td>
<td>0.85</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>Vaginal (80%)</td>
<td>Vaginal (80%)</td>
<td>Vaginal (80%)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Statistical analysis**

The main analysis compared difference scores (vaccination score
minus baseline value) for percentage facial action, percentage
cry duration, and visual analogue scale pain scores among
the groups, by multivariate ANOVA. Univariate, one-way ANOVAs
were carried out only if the multivariate ANOVA was significant
(p<0.05). The pattern of significant differences between pairs of
means was examined by post-hoc comparisons by Duncan's
method. Trend analysis was used to establish the significance of
the rank order among the groups, with the expectation that the
Table 4: Birth characteristics

Emla-treated group would have a pain response intermediate to those of the other groups. Demographic characteristics were compared among groups by ANOVA or χ² test, as appropriate. Temperament scores were analysed by multivariate ANOVA. The strength of linear relations between pain measures and infant variables was assessed by the Pearson or Spearman correlation coefficient as appropriate; correction for multiple correlations was made with the Bonferroni method.

Results

87 (77%) of the 113 eligible infants participated in the study (table 1). Three infants in the uncircumcised group were circumcised after initial contact with the investigator. Two of the three infants were circumcised within 5 days of birth and the other at age 20 days. None of these infants received analgesia for circumcision pain and, therefore, their results were added to the group circumcised with placebo for data analysis.

There were no significant differences among the three groups in any demographic characteristics at the time of vaccination (table 2). Infant temperament was similar in all groups (multivariate ANOVA main group effect; p=0.20); (table 3), as were birth characteristics (table 4). 64 clinics took part in the study. Five (6%) infants were held by a parent during vaccination, and 76 (87%) were vaccinated with a 25-gauge needle. Eight infants were pretreated with Emla for circumcision pain openly in the clinical trial from which they were recruited.

Multivariate ANOVA revealed a significant group effect for difference in pain scores (p<0.001). Univariate ANOVAs (figure) showed significant group effects for percentage facial action (p=0.04), percentage cry duration (p=0.01), and visual analogue scale pain scores (p=0.02). Post-hoc analysis showed that the group circumcised with

<table>
<thead>
<tr>
<th>Correlation coefficient*</th>
<th>% facial action</th>
<th>% cry duration</th>
<th>VAS pain score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postnatal age (days)</td>
<td>-0.10</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>-0.08</td>
<td>-0.09</td>
<td>-0.05</td>
</tr>
<tr>
<td>Time from last feed (min)</td>
<td>-0.09</td>
<td>-0.12</td>
<td>-0.17</td>
</tr>
<tr>
<td>Time from last nap (min)</td>
<td>0.09</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Treated with paracetamol</td>
<td>-0.10</td>
<td>-0.05</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

Table 5: Relation between infant characteristics and pain response

placebo had higher difference scores (p<0.05) than the uncircumcised group for percentage facial action (136.9 vs 77.5%), percentage cry duration (5.1 vs 2.4%), and visual analogue scale pain scores (5.1 vs 3.1 cm). In addition, visual analogue scale pain scores were significantly higher in infants circumcised with placebo than in those circumcised with Emla (5.1 vs 3.1 cm; p<0.05). There was a significant linear trend (p<0.05) in all three outcome measures, with scores increasing from the uncircumcised to the circumcised with placebo group.

The main results were similar when the analysis was repeated by univariate ANCOVAs with vaccination pain score as the outcome and baseline value as the covariate.
Characteristics of the infants, such as age, weight, temperament, ingestion of paracetamol, time of last feeding, and time of last sleep before vaccination, did not correlate significantly with pain response (table 5).

Discussion

This study showed that neonatal circumcision in male infants is associated with increased pain response in vaccination 4–6 months after surgery. The results support our previous finding of a higher pain response in circumcised than uncircumcised male infants during routine vaccination.10

We postulate that circumcision may induce long-lasting changes in infant pain behaviour because of alterations in the infant's central neural processing of painful stimuli. Transmission of noxious afferent input from the periphery (eg, brought about by skin incision) to the spinal cord induces a sustained state of central neural sensitisation or hyperexcitability that amplifies subsequent input from the wound and leads to increased postoperative pain. The specific mechanisms by which noxious peripheral stimulation induces long-lasting central neuronal changes are not yet fully established, but the N-methyl-D-aspartic acid (NMDA) receptor ion-channel complex, excitatory amino acids (eg, glutamate), and C-fibre neuropeptides (eg, substance P) have been implicated. Peripheral noxious stimulation leads to the release of excitatory amino acids and neuropeptides in the dorsal horn of the spinal cord. Activation of the NMDA receptor in dorsal horn neurons produces an increase in intracellular calcium and other secondary messengers, which stimulate protein kinases and new gene expression.20,21

This study was designed to investigate whether premedication with a topical local anaesthetic for circumcision pain would attenuate the pain response to vaccination several months later in circumcised infants. We postulated that Emla would at least partially block nociceptive afferent input originating from the surgical site at the time of circumcision and, therefore, any long-lasting consequences of this input on the central nervous system. The results of the study do not entirely support this hypothesis. Differences in vaccination pain response between infants pretreated with Emla and those given no anaesthesia for circumcision pain were seen for visual analogue scale pain scores, but not for facial action and cry duration. However, there was a significant trend for Emla-treated infants to have an intermediate pain response across all three measures of pain (figure). Although primary afferent injury discharge and subsequent noxious perioperative events contribute to enhanced postoperative pain,2 other factors, such as postoperative inflammatory inputs, may also induce a state of central sensitisation.23 Insufficient afferent blockade during circumcision and in the days that follow surgery may have contributed to central sensitisation in both treated and untreated circumcision groups. Study of the vaccination pain response of infants who had received more effective circumcision pain management (ie, dorsal penile nerve block and adequate postoperative pain management) would be interesting.

Although vaccination pain response displayed by the infants circumcised without analgesia was higher than the uncircumcised infants, this response may not be specific only to pain. The site of injury during vaccination differed from that during circumcision. In addition, vaccination pain measured by facial action and cry duration did not differ significantly between infants circumcised with or without Emla. Although postsurgical central sensitisation (allodynia and hyperalgesia) can extend to sites of the body distal from the wound,24 suggesting a supraspinal effect, the long-term consequences of surgery done without anaesthesia are likely to include post-traumatic stress as well as pain.25 It is, therefore, possible that the greater vaccination response in the infants circumcised without anaesthesia may represent an infant analogue of a post-traumatic stress disorder triggered by a traumatic and painful event and re-experienced under similar circumstances of pain during vaccination.

Factors other than circumcision may account for the observed differences in pain response. For example, there may be differences in genetic attributes, socioeconomic status, and parent-infant interactions between people who have their sons circumcised and those who do not. However, race and socioeconomic status did not differ between groups in this study and there were no observable qualitative differences in the way parents interacted with their infants during the vaccination.

Another possible explanation is that parents of infants who have undergone painful surgical procedures such as circumcision begin to interact differently with their infants compared with parents whose infants have not undergone such procedures. Parents' patterns of behavioural reinforcement may develop so that by the age of 4 or 6 months, circumcised infants may display a heightened pain response to vaccination. Infant temperament was measured to discern differences among groups due to effects of the infants' personalities. However, the revised infant temperament questionnaire did not show any differences in infant behaviour among the groups.

To keep potential bias during data collection to a minimum, we standardised the infants' position before vaccination. Second, we waited for infants to calm down if they were unsettled by the physical examination. Third, each infant was videotaped in his own primary physician's clinic. Finally, videotapes were coded by a research assistant who was not aware of the status of infants in each treatment group or the purpose of the study.

Several other investigators have studied the long-term effects of untreated pain in newborn infants. Fitzgerald and colleagues26 showed that repeated heel lancing may induce a state of hypersensitivity in pain response, and that this atypical response can be prevented by pretreatment with Emla. Grunau and colleagues27,28 found that children born prematurely have a tendency to somatise and interpret pictures of pain-producing situations differently from other children. Finally, long stays in hospital and repeated medical procedures in the perinatal period have been proposed as factors affecting long-term cognitive and motor deficits seen in low-birthweight infants.29,30

The results of this study are consistent with studies of pain response in animals and behavioural studies in humans showing that injury and tissue damage sustained in infancy can cause sustained changes in central neural function, which persist after the wound has healed and influence behavioural responses to painful events months later. Pretreatment and postoperative management of neonatal circumcision pain is recommended based on these results. Investigation of the neurological basis of these effects is warranted.
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References