

were available, and intravenous methylene blue, the antidote, was to hand.

Our trial was abandoned after 22 infants had been entered. Obtaining fully informed consent was usually difficult due to mothers' fears of methaemoglobinaemia and lack of reports of the safety of EMLA in neonates, so recruitment was too slow to complete the trial within a reasonable length of time. Randomisation had resulted in five babies given EMLA and 17 placebo.

No baby who received EMLA became blue, desaturated, or developed symptoms, so five neonates were treated safely with EMLA on our fairly stringent regimen. We would be interested to know how the Edinburgh team coped with explaining and countering the possible hazards of methaemoglobinaemia which do not seem well researched in neonates. Knowledge of the safe use of EMLA in their 28 neonates as well as our precautions in five babies, and Koren's suggestions of unofficial safe use should greatly facilitate obtaining informed consent in future trials.

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- 1 McIntosh N, van Veen L, Brameyer H. Alleviation of heel prick in preterm neonates. *Arch Dis Child* 1994; 70: F177-81.
- 2 Fitzgerald M, Millard C, McIntosh N. Cutaneous hypersensitivity following peripheral tissue damage in newborn infants and its reversal with topical anaesthesia. *Pain* 1989; 39: 31-6.
- 3 Koren G. Use of the eutectic mixture of local anesthetics in young children for procedure-related pain. *J Pediatr* 1993; 122 (suppl): 30-5.

Dr McIntosh comments:

In our studies using EMLA cream we were attempting to reduce pain and distress (apparently unsuccessfully) in newborn infants receiving heel pricks. The parents were informed that EMLA cream had not been used other than in our own study on neonates, but that it was commonly used and with no problem in older children. We knew about the possibility of methaemoglobinaemia but at the time of starting the study there was only one report of this problem in a child who was also receiving a sulphonamide, so we did not believe that we ought to inform the parents of this specific but theoretical hazard.

Lipid peroxidation as a measure of oxygen free radical damage in the very low birthweight infant

EDITOR,—We read with interest the paper by Inder *et al* on lipid peroxidation as a measure of oxygen free radical damage in preterm infants.¹ They showed a rise in malondialdehyde detected by the thiobarbituric acid (TBA) test over the first week which was significantly greater in those infants developing chronic lung disease. We have also used the TBA test to detect lipid peroxidation in 131 very preterm infants during the first seven days after birth. Concentrations rose from a median of 2.13 $\mu\text{mol/l}$ (1.63-2.77 range) on day 1 to 3.27 $\mu\text{mol/l}$ (2.49-4.48) on day 7 in those not developing chronic lung disease and from 2.07 $\mu\text{mol/l}$ (1.16-2.98) to 3.77 $\mu\text{mol/l}$ (2.6-4.21) in the 40 infants who developed chronic lung disease. No significant difference was observed. It is of interest that our values for the TBA test were about 30 times lower than those of Inder *et al*, in keeping with other published values for the test.² We used a fluorimetric method, but the HPLC technique used by Inder *et al* generally gives lower values than the fluorimetric method.³ Until these differences are explained, we cannot accept the authors' findings as evidence for lipid peroxidation in very preterm infants.

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- 1 Inder TE, Graham P, Sanderson K, Taylor BJ. Lipid peroxidation as a measure of oxygen free radical damage in the very low birthweight infant. *Arch Dis Child* 1994; 70: F107-11.
- 2 Richard MJ, Guiraud P, Meo J, Favier A. Physiological and pathological values of malondialdehyde (MDA) in plasma as determined by HPLC. *J Chromatogr* 1992; 577: 9-18.
- 3 Wong SHY, Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW. Lipoperoxides in plasma as measured by lipid-chromatographic separation of malondialdehyde thiobarbituric acid adduct. *Clin Chem* 1987; 34: 214-20.

Dr Inder and coauthors comment:

We are grateful for the query from Professor Cooke and colleagues which identified an unfortunate calculation error in our malondialdehyde-thiobarbituric acid (MDA-TBA) values that occurred during the conversion of

our standard values in ng/ml to $\mu\text{mol/l}$. Due to this error the published MDA-TBA values were too high by a factor of 60, and should read for cord blood in full term infants (n=48) mean (SD) 1.05 $\mu\text{mol/l}$ (0.16); premature infants without chronic lung disease (n=6) from cord blood concentrations of 1.19 (0.1) $\mu\text{mol/l}$ to 1.72 (0.1) $\mu\text{mol/l}$ at 7 days and in premature infants with chronic lung disease (n=16) from cord blood concentrations of 1.42 (0.1) $\mu\text{mol/l}$ to 2.66 (0.2) $\mu\text{mol/l}$ at 7 days. These values are approximately half those found by Cooke *et al* in his premature infants. However, the significance of the raised MDA-TBA values in premature infants with chronic lung disease is unchanged. Why did our assay detect a significant difference in MDA-TBA concentrations? The key issue relates to the specificity of thiobarbituric assays for malondialdehyde as indicators of lipid peroxidation. Both the method we used¹ and the method of Wong *et al*,² use HPLC to measure MDA-TBA, which eliminates inaccuracies due to interfering chromogens. However, there are several important differences. In our assay, no EDTA is added, plasma lipids are extracted before analysis, and ferric chloride (FeCl_3) plus butylated hydroxytoluene are added before heating with TBA. The rationale for adding FeCl_3 was to promote efficient breakdown of lipid hydroperoxides to MDA,³ but we are not sure that this is its only mode of action. The Wong method uses whole plasma and is thought to measure primarily protein bound MDA. Thus, although both are considered to be indicators of lipid peroxidation the two methods may clearly be measuring two different parameters. Further ongoing research in our premature infants continues to support the findings we have published. However, to understand the true nature of the MDA-TBA product measured, more specific analytical measures of lipid peroxidation products are awaited.

- 1 Wade CR, Van Rij AM. Plasma thiobarbituric acid reactivity: reaction conditions and the role of iron, antioxidants and lipid peroxy radicals on the quantitation of plasma lipid peroxides. *Life Sci* 1988; 43: 1085-93.
- 2 Wong SHY, Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clin Chem* 1987; 33: 214-20.
- 3 Guttendge JMC, Quinlan GJ. Malondialdehyde formation from lipid peroxides in the thiobarbituric acid test: the role of lipid radicals, iron salts and metal chelators. *J Appl Biochem* 1983; 5: 293-9.