BIOLOGICAL EFFECTS, IN PREMATURE INFANTS, OF ALPHA-LINOLENIC ACID-ENRICHED MILK FORMULAS (RAPESEED OIL): A MULTICENTRIC STUDY

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ABSTRACT

The present multicentric study was designed to investigate the interest of LEAR (Low Erucic Acid Rapeseed) oil as source of α-linolenic acid (α-LnA) for enrichment of regular milk formula (RF) for premature infants. Plasma and red blood cell (RBC) fatty acid composition was evaluated in 88 premature infants fed either RF or enriched formula (1.95% vs 0.55% α-LnA) or human milk. The results concluded to the high availability of this acid, its good conversion into higher n-3 derivatives : EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid) (the latter to a lower extent) as regards the data in plasma and RBC phospholipids. Since DHA is known to play a major role in brain and in retina development and function, LEAR oil appears as a useful tool for supplying premature newborns with αLnA, the precursor of the n-3 serie.

INTRODUCTION

The possibility of long chain n-3 polyunsaturated fatty acids (LC-PUFA) deficiency in premature newborns fed exclusively formulas was raised recently and in the n-3 serie the role of DHA was specially pointed out. DHA levels have been found to be significantly lower in the phospholipid FA of the cerebral cortex of premature infants fed with formula contrary to those fed breast milk (FARQUARSON et al). This was associated with visual acuity disturbancy (UAUY et al ; MAKRIDES et al.) and lower intelligence quotient (LUCAS et al). Indeed, some studies have confirmed the role of DHA in light phototransduction in retina (DRATZ et al, BROWN et al).

The absence of PUFA of the n-3 series in regular formulas (RF) would appear to be nutritionnally detrimental in early human development and particularly in premature infants because 1) adipose tissu stores are lower than in term infants, 2) desaturase hepatic activities are probably more immature (POISSON et al.) and 3) the phase of rapid brain growth can be disturbed by deprivation of maternal and placental supply after premature birth. When breast-feeding is impossible, LC-PUFA should be supplied by formula feeding. This can be done either by supplementation with fish oils containing EPA and DHA or through metabolic synthesis from their essentiel C18 precursor fatty acid provided by vegetable oils. This second possibility was investigated in the present study using LEAR (Low Erucic Acid (=1,2%) Rapeseed) oil to supply regular milk formula (RF) with α-linolenic acid (α-LnA :1.95% vs 0.55%) obtained by partial substitution of fats with a french LEAR oil.

EXPERIMENTAL

Both formulas, RF and EF, were obtained from Nutripharm (Levallois-Perret, France). The α-LnA enrichment in EF respected the ratio linoleic acid/α-LnA = 6. α-LnA was provided by LEAR oil (Codex Alimentarius, Canadian General Standard Board, 1987). The study included 88 premature infants (32 wk mean gestational age (GA) and 1621g mean weight at birth). All the infants were admitted to the Neonatal Departments in Bordeaux, Caen and Montpellier (France); 25 were fed with human milk (HM) provided from the lactarium, 32 completed the formula group control (RF) and 32 were fed with the enriched formula(EF). For the 3 groups, the enteral feeding began 3 days after birth and progressively substituted the parenteral feeding. 3 blood samples were obtained at the second and the 15th day of milk or formula feeding (D2 and D15 respectively) and the third one at discharge from the hospital i.e. at 37 wk GA (about 4-5 wks of enteral feeding).Total lipids of 200µl of plasma and 200µl of

RBC were extracted with 3ml [chloroform-methanol (2:1, vol/vol) and butylated hydroxytoluene as anti-oxydant]. Plasma and RBC lipid classes were separated by thin-layer chromatography and FA methylesters obtained by transesterification with 19N sulfuric acid in methanol (1:19, vol/vol) 95°C, 35mn and analysed by gas-liquid chromatography with a CP Sil 88 capillary column, Delsi DI 200 gas chromatograph, hydrogen as carrier gas and an Enica 10 as integrator.

RESULTS AND DISCUSSION

The main results expressed in weight percent of all the fatty acids detected are presented in the following table.

D 2		RF	EF	RF vs EF	HM
C18:3 (n-3)	TG	0.42 (n=27)	0.94 (n=30)	p<0,0001	0.40 (n=23)
C20:4(n-6)	PL	10.83 (n=29)	9.69 (n=30)	n.s.	11.18 (n=23)
	PE	14.86 (n=26)	15.70 (n=23)	n.s.	16.51 (n=22)
C20:5(n-3)	PL	0.20	0.44		0.36
	PE	0.14	0.40		0.51
C22:6 (n-3)	PL	2.54 (n=29)	2.52 (n=30)	n.s.	2.80 (n=23)
	PE	3.15 (n=28)	3.26 (n=29)	n.s.	3.67 (n=22)

TG, plasma triglycerides
PL, plasma phospholipids
PE, erythrocyte phosphatidylethanolamines

RF, regular formula
EF, enriched formula
HM, human milk

D 1 5		RF	EF	RF vs EF	HM
C18:3 (n-3)	TG	0.44 (n=30)	1.29 (n=30)	p<0.0001	0.54 (n=25)
C20:4(n-6)	PL	7.53 (n=32)	6.88 (n=30)	p<0.05	9.63 (n=25)
	PE	14.30 (n=31)	14.43 (n=30)	n.s.	14.42 (n=25)
C20:5(n-3)	PL	0.15	0.55		0.40
	PE	0.32	0.40		0.40
C22:6 (n-3)	PL	1.54 (n=32)	2.20 (n=30)	p<0.0001	2.81 (n=25)
	PE	2.54 (n=31)	3.02 (n=30)	n.s.	2.66 (n=25)
decrease PE-DHA*		-0.61	-0.24	p<0.05	

* difference between D2 and D15

37 W		RF	EF	RF vs EF	HM
C18:3 (n-3)	TG	0.59 (n=26)	1.34 (n=25)	p<0.0001	0.70 (n=18)
C20:4 (n-6)	PL	7.99 (n=26)	7.08 (n=25)	p<0.01	10.10 (n=18)
	PE	14.86 (n=26)	15.70 (n=23)	n.s.	18.24 (n=18)
C20:5(n-3)	PL	0.20	0.47		0.28
	PE	0.23	0.50		0.34
C22:6 (n-3)	PL	1.58 (n=26)	2.27 (n=25)	p<0.0005	2.77 (n=18)
	PE	2.41 (n=26)	3.14 (n=23)	n.s.	4.08 (n=18)

The ratio linoleic acid/α-LnA = 6/1 was retained in EF as the best able to raise DHA without significantly decreasing AA levels in plasma and RBC phospholipids in preterm infants (CLARK et al). In these conditions :

1) The effect of 18:3(n-3) complementation resulted in a 2.5 fold higher level of 18:3(n-3) in the TG as soon as the 2nd day and till 37 W of GA. This demonstrates the excellent incorporation in plasma TG of this acid originating from the colza (or rapeseed) oil experimented and its good availability for metabolism.
2) The bioconversion of α-LnA seems to be efficient as regards the higher level of DHA in the PL of the enriched formula (EF) group vs the regular formula (RF) group at D15 ($p<0.001$) and at 37 W of GA ($p<0.005$). Again, differences are observed for DHA level in the PE of RBC (2.4% vs 3.14%DHA at 37 W in PE for RF vs EF groups respectively but without statistical significance).
3) The decrease of DHA in PE (-0.24%) observed in the EF group was significantly lower ($p<0.05$) than in the RF group.
4) Though both EF and RF groups received the same vit E supplementation (two fold the Europ. Comm. Recommandation) plasma vit E was higher in HM and EF groups (11.1 ± 1.4 and 10.8 ± 1.3 μg/ml) than in RF group (6.2 ± 0.9) at D15 ($p < 0.02$) and ($p < 0.05$) at 37W.

The present data suggest an efficient conversion of the α-LnA from the enriched formula into its higher LC-PUFA derivatives and their incorporation in plasma and RBC phospholipids.

In the EF group the supplementation resulted in complete maintenance of EPA at the level observed in the HM group whereas the DHA levels were only partly preserved.This difference could result from 1) delay of the insertion of DHA in the membranes of the RBC, and 2) lower conversion of EPA into DHA by the so called "Δ4 desaturase" system. In the EF group, AA levels in PL were weakly but significantly lower than in PL of RF group. This could result from the known incidence of the neoformed EPA on the Δ5 desaturase of the n-6 patway (NASSAR et al.), an effect which has been shown to be detrimental to growth. This suggests that it would be advisable to limit the α-LnA supply in the formula to 0.80 % of total calories. However it should be observed that in the present study AA in RBC membranes was not affected.

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