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Simulated amniotic fluid–like solution given enterally to neonates after obstructive bowel surgeries: A randomized controlled trial

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ABSTRACT

Objectives: Withholding postoperative feeding is common in neonates recovering from surgeries for congenital abnormalities of the gastrointestinal tract (GIT), which leads to prolonged exposure to total parenteral nutrition, intestinal atrophy, and feeding intolerance. Because amniotic fluid plays a significant role in fetal gut maturation and development, the aim of this study was to test a hypothesis suggesting that feeding tolerance could be improved in neonates recovering from surgeries for congenital obstructive bowel abnormalities by enteral administration of simulated amniotic fluid-like solution given enterally (SAFE) containing recombinant human granulocyte colony-stimulating factor and erythropoietin.
Methods: This prospective, double-blind, randomized, placebo-controlled trial was conducted with 40 late preterm/term neonates recovering from GIT surgeries. Neonates were randomly divided postoperatively into two groups: 20 neonates received the test solution (SAFE group) and 20 neonates received distilled water (placebo group) with a gestational age range (34.3–40.4 versus 34–40 wk, respectively) and mean gestational age (37.10 ± 1.68 versus 36.90 ± 1.83 wk, respectively). Treatment was started postoperatively and the test solution (or distilled water) was discontinued when daily enteral intake reached 100 mL/kg.
Results: The study group showed better feeding tolerance as demonstrated as reflected by an earlier achievement of 50, 100, 120, and 150 mL/kg enteral feeding per day with a higher enteral caloric intake on day 7 post SAFE administration and a higher rate of weight gain ($P < 0.05$ for all).
Conclusion: Enteral administration of SAFE may improve postoperative feeding tolerance, enteral caloric intake, and weight gain.

Introduction

Feeding intolerance is common in neonates recovering from surgeries for congenital abnormalities of the gastrointestinal tract (GIT) such as intestinal atresia, omphalocele, gastroschisis, or imperforate anus [1]. Feeding intolerance in these patients is multifactorial, but one of the important reasons is congenital maldevelopment of the intestinal villi. Each congenital anomaly involves different degrees of dysmorphic mucosa, but all of them lack normal histologic architecture with blunted villi and with disorganized and shallow crypts [2].

The human fetus swallows >200 mL/kg of amniotic fluid daily, which is essential for normal intestinal development. Amniotic fluid plays an important role in fetal gut maturation and development [1]. Several growth factors have been identified in the amniotic fluid including epidermal growth factor and insulin-like growth factor 1, in addition to erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF); many of them bind to receptors on the luminal surface of villous enterocytes and promote proliferation of fetal intestinal cells [3]. EPO and G-CSF have the practical advantage of being available as sterile human recombinant factors in quantities sufficient enough to be used in clinical trials [1].

In the dynamic milieu of the developing gut, several factors have been implicated in the pathogenesis of necrotizing

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enterocolitis (NEC). Among those mechanisms, withholding postoperative feeding for an extended period has been suggested to lead to more intestinal atrophy secondary to lack of trophic hormone stimulation and luminal starvation. These gastrointestinal changes can disrupt the barrier function of the GIT and increase the bacterial translocation, impair immune function, and lead to the development of NEC [4].

Several studies targeted the effect of administration of simulated amniotic fluid-like solution given enterally (SAFE) in the prevention of feeding intolerance and NEC in preterm and very low birth weight neonates in general [5–8]; however, its effect on neonates recovering from gut surgeries has not been sufficiently examined. Therefore, this study was conducted to test a hypothesis suggesting that feeding tolerance could be improved in neonates recovering from surgeries for congenital obstructive bowel abnormalities by enterally administered intestinal growth factors included within the simulated amniotic fluid-like solution. The secondary aim was to decrease nil per os (NPO) days and decrease the need for total parenteral nutrition (TPN) to avoid its complications and cost.

Patients and methods

This prospective, double-blind, randomized controlled clinical trial was conducted with 40 late preterm and term neonates recovering from GIT surgeries for congenital bowel abnormalities at the neonatal intensive care units (NICUs), Ain-Shams University Hospitals. Neonates with operable congenital obstructive bowel abnormalities; esophageal atresia with or without fistula, duodenal atresia, ileojejunal atresia, colonic atresia, and Hirschsprung disease were included. Exclusion criteria included hemodynamically unstable babies, those exposed to hypoxic insult, prior use of cytokine or intravenous immunoglobulin, and those with any contraindication to enteral feeding such as intestinal perforation or leakage at the anastomosis site. The study was approved by the ethical committee of the Pediatrics department, Ain-Shams University and informed consents were taken from parents before enrollment. This trial was registered in ClinicalTrials.gov. Reporting of the study conforms to the CONSORT 2010 statement [9] (Supplementary Table 1).

Randomization and blinding

Neonates were randomly subdivided postoperatively into two groups with 20 neonates received the test solution (SAFE group) and 20 neonates received plain distilled water without any additives (placebo group). Distilled water is an inert substance that does not have any pharmacologic effect on the patient, which is why it is used as a placebo in different blind clinical drug trials [7,10].

Neonates were randomized according to simple randomization technique; sequential-order sequence was maintained within the investigational drug pharmacy with allocation concealment by an opaque sequentially numbered sealed envelope. Blinding included the parents of the studied neonates, the attending neonatologist, data collectors, study nurses, outcome adjudicators, and laboratory personnels. The pharmacist was aware of the nature of the intervention but played no other part in the conduct of the trial. Upon enrollment of a neonate, the pharmacist sent eight syringes to the NICU, each containing 2.5 mL/kg of SAFE/placebo. The syringes were covered in pharmacy bags to mask the contents.

Initial assessment

All the included neonates were subjected to comprehensive history taking, thorough clinical examination, and routine neonatal care. The clinical characteristics of the SAFE group were comparable with those of the placebo group with no significant difference between both groups regarding mean gestational age (37.10 ± 1.68 versus 36.90 ± 1.83 wk, respectively), mean birth weight (2.69 ± 0.46 versus 2.42 ± 0.57 kg, respectively), male sex (50 versus 60%, respectively), median Apgar score at 1 min (7 versus 7), median Apgar score at 5 min (8 versus 8), vaginal mode of delivery (30 versus 25%, respectively), as well as history of maternal diseases.

The SAFE group included two cases of esophageal atresia without fistula, four esophageal atresia with fistula, seven duodenal atresia, four jejunal atresia, two ileal atresia, and one colonic atresia. The placebo group included one case of esophageal atresia without fistula, five esophageal atresia with fistula, eight duodenal atresia, two jejunal atresia, one ileal atresia, one duplicating duodenal cyst, and two Hirschsprung disease.

Laboratory investigations were done at baseline (day 0) and on day 7 post-treatment/placebo to detect any adverse effects of treatment. Labs included complete blood count with differential leukocytic count using Sysmex XT1800-I (Sysmex, Kobe, Japan); C-reactive protein quantitative assay using Latex agglutination slide test (Avitex CRP kit, Omega Diagnostic Limited, Scotland, United Kingdom). Liver and kidney function tests performed on Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany), and serum electrolytes in addition to blood culture pre- and postoperatively. Patients also had baseline evaluations in the first 72 h of life that included plain x-ray abdomen (erect and supine), pelvic and abdominal ultrasounds, as well as echocardiographic assessment. These were repeated whenever clinically indicated.

Preparation and administration of the study drug

The SAFE was prepared by a specialized pharmacist using a sterile technique as previously reported [11]. The solution contained 115 mEq/L sodium chloride, 17 mEq/L sodium acetate, 4 mEq/L potassium chloride, 225 ng/mL recombinant human granulocyte colony stimulating factor (rhG-CSF; Geneleukin 300 μg/mL,

Table 1
Feeding and clinical characteristics of the two groups of neonates

Variable	SAFE group (n = 20)	Placebo group (n = 20)	t/χ^2/Z test	*P*-value
Age of diagnosis (d), mean ± SD	4.10 ± 6.91	2.25 ± 4.24	1.020	0.314
Age when operated (d), mean ± SD	9.00 ± 8.38	5.65 ± 5.14	1.524	0.136
Age feeding started (postoperative days), median (IQR)	9 (11)	6.5 (4)	−0.76	0.447
Type of milk, n (%)				
Human	3 (15)	4 (20)	0.524	0.770
Formula	10 (50)	11 (55)		
Mixed	7 (35)	5 (25)		
Time to achieve 20 mL·kg·d^{-1} (postoperative days), median (IQR)	11 (13.75)	11.5 (6)	−0.41	0.968
Time to achieve 50 mL·kg·d^{-1} (postoperative days), median (IQR)	12.95 (13.5)	14.65 (7.75)	−2.430	0.047
Time to achieve 100 mL·kg·d^{-1} (postoperative days), median (IQR)	15.3 (13.75)	17.7 (9.75)	−3.101	0.032
Time to achieve 120 mL·kg·d^{-1} (postoperative days), median (IQR)	16.5 (13.5)	18.5 (11)	−2.744	0.041
Time to achieve full intake (postoperative days), median (IQR)	19 (20.75)	21.5 (13.25)	−2.001	0.048
When TPN stopped (postoperative days), median (IQR)	16.5 (13.5)	18.5 (11)	−2.732	0.042
Rate of weight gain (g·kg BW·d^{-1}), median (IQR)	8 (5.75)	4 (8.25)	−2.133	0.033
Total days of hospitalization, median (IQR)	30.50 (18.50)	25.50 (25.25)	−0.826	0.414
Postoperative days of hospitalization, median (IQR)	20.50 (20.50)	22.50 (23.25)	−0.135	0.892
Mortality, n (%)	2 (10)	5 (25)	1.558	0.212

BW, birth weight; IQR, interquartile range; SAFE, simulated amniotic fluid-like solution given enterally.
Data expressed as mean ± SD where Student's *t* test was applied for comparisons or as median and IQR (the difference between 25th and 75th centiles) where Mann–Whitney test (*z*) was used or as number (%) where Chi-square test (χ^2) was applied for comparisons.

Shandong Geneleuk biopharmaceutical Co. Ltd., P.R. China), and 4400 mU/mL recombinant human erythropoietin alfa (rhEPO; Epoetin 2000 IU/ mL, SEDICO Pharmaceuticals Co., Egypt). Human albumin (5%) was added to the solution (final concentration of albumin was 0.05%). The daily dose of the enteral study solution was 20 mL/kg containing 4.5 μg/kg rhG-CSF and 88 IU/kg rhEPO to mimic the same amount of growth factors ingested by a fetus swallowing 200 mL/kg of amniotic fluid daily, according to Christensen et al. [6]. The control group enterally received plain distilled water without any additive as a placebo.

Aliquots of the SAFE and placebo were frozen until use at 2°C to 8°C according to manufacturer instructions and as described by Canpolat et al. [12]. Separate aliquots (10 mL) were labeled "priming" to be pushed through an orogastric tube before insertion of the test solution to reduce its binding to the plastic tubing. Upon enrollment of a neonate, a full daily dose and a priming syringe were thawed, labeled, divided into eight equal amounts in separate opaque aliquots and warmed to room temperature by placing them near the patient for 10 to 15 min to be administered and recorded by bedside nurse every 3 h.

Test solution (or placebo) was administered either by orogastric tube, oral route, or gastrostomy tube, with the initiation of trophic feeding mixed with milk to prevent any adhering of EPO to feeding catheter [13]. Test solution (or placebo) was administered for a maximum of 7 d or was discontinued when the enteral intake reached 100 mL/kg daily (20 mL/kg of experimental solution plus 80 mL/kg of milk), whichever came first.

Feeding protocol

Neonates started enteral (gavage) feeding as soon as it was deemed appropriate by the pediatric surgeon and attending neonatologist. Feeding regimen was dictated by local guidelines for such cases. The feeding policy of the unit is to start daily trophic feeding with 10 to 20 mL/kg and progressed with 10 to 20 mL/kg as long as tolerated (judged by the attending neonatologist). Feeding took place every 3 h and volumes increased as clinically tolerated. Expressed breast milk (EBM) was encouraged, and formula feeds were used if expressed breast milk was not available. Feeding policy did not differ during the study period.

Feeding intolerance is defined with the presence of any of the following that lead to interruption of the enteral feeding: gastric residual exceeding 50% of the previous feed volume, increased abdominal girth by ≥2 cm between feedings, emesis, diarrhea, visible blood in the stool not otherwise explained, or abnormally enlarged bowel loops found on physical examination or abdominal radiograph [14].

Outcome measures and endpoint of the study

The following nutritional data were recorded: day of successful start of enteral treatment; type of milk used; duration until establishing one-third (50 mL·kg·d^{-1}), two-thirds (100 mL·kg·d^{-1}), and full enteral feeding (150 mL·kg·d^{-1}); time of TPN discontinuation; day of onset of weight gain; daily weight gain expressed as g/kg; daily enteral caloric intake (kcal/kg); incidence of NEC stage 2 or worse according to Bell's modified criteria for NEC staging [15]; surgery for NEC during the study period; NEC-related deaths; duration of hospitalization; hospital readmissions; and adverse effects of treatment (if any) for the duration of the study and for the ensuing week.

Statistical analysis

The sample size was calculated using Stata version 11 (StatCorp, College Station, TX, USA), setting α error at 5% and power at 80%. Results from a study by El-Ganzoury et al. [7] reported that among the placebo group, the mean duration for weight gain was 14.9 ± 6.5 compared with 10.1 ± 4.1 for the treatment group. Calculation according to these values produced a required minimal sample size of 16 participants per group.

Data were analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). One sample Kolmogorov–Smirnov test was used to examine the normality of data distribution. Qualitative data were presented as number and percentages; whereas quantitative variables were described as mean and SD or median and interquartile range (IQR; the difference between the 25th and 75th centiles). Comparison between two groups with qualitative data was done using χ^2 test. Fisher exact test was used instead of the χ^2 test when the expected count in any cell was found <5. Comparison between two independent groups regarding quantitative data with parametric distribution was done by using the independent *t* test; whereas the comparison of quantitative data with non-parametric distribution among two groups was done using the Mann–Whitney test. $P < 0.05$ was considered the cut-off value for significance in all analyses.

Results

All of the neonates studied tolerated the received treatment solution well without side effects that could be attributed to the drug intake. The age at start of SAFE or placebo was 16.95 ± 10.37 versus 14.35 ± 6.82 d, respectively ($P = 0.091$). Twelve neonates did not complete 7 d of treatment solution (or placebo) owing to earlier achievement of 100 mL/kg of milk per day; they included nine neonates in SAFE group and three in the placebo group with no significant difference between both groups regarding the mean duration of administration (6.50 ± 3.40 versus 8.05 ± 3.46 d, respectively; $P = 0.615$).

The study group showed earlier achievement of 50, 100, 120 mL/kg and full enteral feeds; earlier time to stop TPN with a higher rate of weight gain postoperatively after SAFE administration ($P < 0.05$ for all; Table 1).

Figure 1 shows a significantly higher enteral caloric intake on day 7 of administration of SAFE compared with placebo ($P = 0.037$); whereas no significant difference was detected between either group regarding the enteral caloric intake baseline and during SAFE or placebo intake.

A significantly higher incidence of abdominal distention was found among the placebo group than among the SAFE group ($P = 0.011$); whereas the incidence of vomiting, high gastric residual volume, and NPO days showed no significant difference between either group (Fig. 2). There were no reported cases of NEC in either group, even though 18 neonates (40%) in the SAFE group and 15 (75%) in the placebo group received packed red blood cell transfusions.

Regarding laboratory data, there was no significant difference between the groups concerning white blood cell (WBC) count,

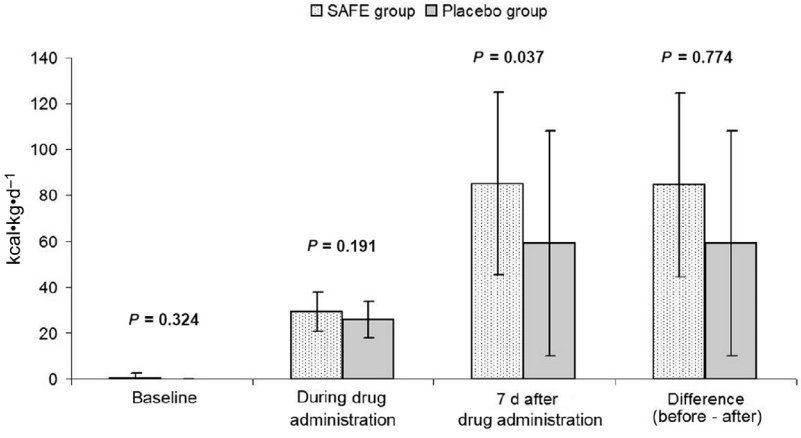

Fig. 1. Enteral caloric intake in the two groups in relation to SAFE/placebo administration. SAFE, simulated amniotic fluid-like solution given enterally.

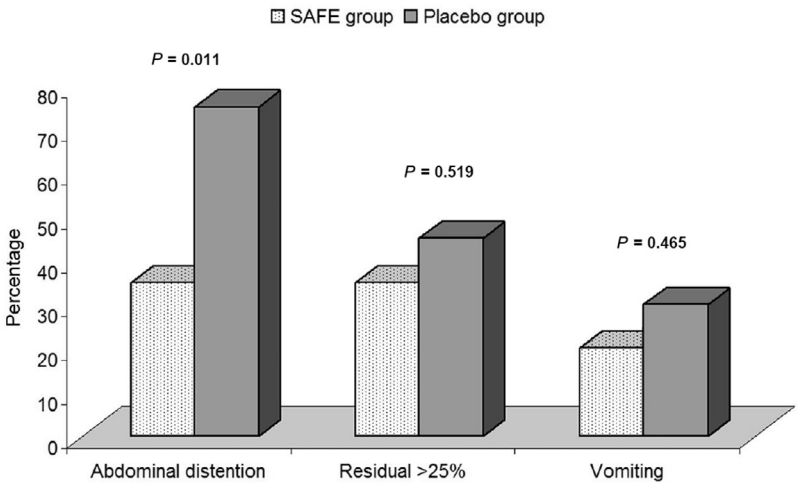

Fig. 2. Manifestations of feeding intolerance among SAFE and placebo groups. SAFE, simulated amniotic fluid-like solution given enterally.

hemoglobin (Hb), hematocrit (Hct), and platelet counts measured at baseline and on day 7 ($P > 0.05$ for all). Upon comparing pre- and post-treatment/placebo data, there was a significant decrease in Hb (12.93 ± 2.09 versus 11.79 ± 1.96, respectively, $P < 0.001$) and Hct values (38.40 ± 5.25 versus 35.63 ± 4.97, respectively, $P = 0.002$) on day 7 after SAFE administration compared with pre-treatment values.

In view of the secondary outcome measure, Table 1 shows no significant difference between the groups regarding the total duration of hospital stay ($P = 0.414$), postoperative duration of hospitalization ($P = 0.892$), and mortality rate ($P = 0.212$).

Discussion

Swallowing amniotic fluid and its growth factors is impaired in a fetus with congenital bowel atresia. Normally, these growth factors when swallowed by the fetus bind to receptors on the luminal surface of villous enterocytes [3,16]. It is postulated that it is the lack of such binding results in dysmorphic changes in the mucosa [2] and that a limitation in amniotic fluid swallowing could constitute at least part of the explanation for postoperative feeding intolerance [1].

We hypothesized that feeding tolerance could be improved in neonates recovering from surgeries for congenital obstructive bowel abnormalities by providing them with physiologic quantities of SAFE that contained cytokine concentrations comparable with what they ingested from amniotic fluid in utero.

All studied neonates tolerated the received treatment well without side effects that could be attributed to drug intake such as skin rash, emesis, blood pressure instability, worsening of respiratory distress, or worsening of liver or kidney function during the days the test solution was given.

In view of the effect of SAFE on postoperative feeding tolerance in these neonates, we demonstrated that the SAFE group showed better feeding tolerance as reflected by an earlier achievement of 50, 100, 120 mL/kg and full enteral feeds and earlier time to stop TPN with a higher rate of weight gain postoperatively after SAFE administration. Moreover, the SAFE group showed a lower incidence of abdominal distention than the placebo group with no reported cases of NEC in either group.

In this context, Juul et al. [17,18] recognized rhEPO for its anti-apoptotic, developmental, and trophic actions on fetal small bowel mucosa. Furthermore, Canpolat et al. [19] examined the effects of recombinant human G-CSF on intestinal cells in hypoxia-induced experimental NEC in rats and concluded that G-CSF has a protective effect on intestinal damage. Barney et al. [20] suggested that a sterile, isotonic solution, patterned after human amniotic fluid with its growth factors, will be helpful in conditions where the intestinal mucosa has undergone atrophic changes, amenable to growth- factor treatment. The trophic effect of that solution on intestinal villi would facilitate advancement to full enteral feedings [1].

Barney et al. [20] observed that recipients of this solution tolerated milk feedings better and achieved higher enteral caloric intake than they had done during the preceding days and that this effect persisted beyond the days the solution was given. It was documented that neonates in the test solution group had fewer NPO days than those in the control group [6]. In addition, El-Ganzoury et al. [7] showed a significant decrease in time to achieve half, three-fourths, and full enteral feeding and concluded that enteral administration of rhG-CSF or rhEPO improves feeding outcome and decreases the risk for NEC.

In the current analysis, we demonstrated that there was no significant difference in WBC count, Hb concentration, and Hct between the groups 7 d post-treatment; this supports the hypothesis that enterally given G-CSF and EPO have a local action on the GIT with no systemic effect. In an additional assessment of lack of systemic absorption of the examined growth factors, we found no significant difference between pre- and post-treatment WBC, Hb, and Hct values.

These results support those of Calhoun et al. [21], who demonstrated no change in plasma G-CSF concentration 2 and 4 h after the administration of a large enteral G-CSF dose. The dose of rhG-CSF they administered (100 μg/kg) was 100 times the normal daily fetal enteral intake of G-CSF consumed by swallowing amniotic fluid. They reasoned that if no G-CSF was detected in the plasma of neonates after swallowing a pharmacologic dose, absorption was unlikely to occur after swallowing physiologic quantities.

Moreover, Juul and Christensen [22] randomly assigned a study in neonates with the administration of 1 dose of rhEPO 1000 U/kg and 1 dose of placebo with measurement of serum EPO concentrations at baseline, 2 and 4 h after study drug administration. They documented that EPO concentrations and Hct values measured at 2 and 4 h after enteral administration of rhEPO did not differ from controls regardless of the duration of therapy. The researchers stated that enterally dosed rhEPO was not absorbed in amounts sufficient to promote increased erythropoiesis. Instead, it was associated with trophic effects in the developing neonatal rat intestine.

The limitation of the present study was the low percentage of absolute breast milk feeding and the use of formula feeds either alone or mixed with breast milk. Nevertheless, feeding tolerance was improved with the achievement of higher weight gain and higher enteral caloric intake as well as earlier TPN discontinuation, denoting that adding growth factors to the formula actually simulated breast milk with its trophic effects on the intestinal mucosa.

Conclusion

This interventional clinical study suggested that SAFE might prevent postoperative malnutrition and feeding intolerance. The next step is to investigate the use of the amniotic fluid itself as a therapeutic method in larger randomized trials to elucidate its safety and efficiency, being a biological fluid, before clinical application on a wider scale.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.05.001.

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