REGULAR ARTICLE

Antisecretory factor effectively and safely stops childhood diarrhoea: a placebo-controlled, randomised study

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ABSTRACT

Aim: We studied the response to high doses of egg yolk containing antisecretory factor (B221®, Salovum®) in young children with acute diarrhoea, presenting to the Children's Hospital, Lahore, Pakistan.

Methods: In a randomised, placebo-controlled trial, 36 children aged 7 to 60 months with acute diarrhoea of unknown aetiology, with mild-to-moderate dehydration, were randomised to the Salovum® or placebo groups. Initially, 16 grams of Salovum® or ordinary egg yolk (placebo) mixed in oral rehydration salts was given, followed by 8 g every 5 h until recovery. The number and consistency of stools were recorded.

Results: The two groups were comparable in age, gender, duration of diarrhoea, hydration and nutritional status, although the proportion with watery stools was higher in the Salovum® group ($p = 0.04$). Reduction in the frequency of stools was seen at 7 versus 18 h ($p < 0.0001$) and normalising of stool consistency was 10 versus 18 h, $p < 0.03$) in the Salovum® and placebo groups. The overall effect was 35 versus 70 h in the two groups ($p = 0.001$). No side effects were reported.

Conclusion: High doses of AF in the form of Salovum® effectively and safely reduce childhood diarrhoea of a likely broad aetiology.

INTRODUCTION

Antisecretory factor (AF) is an endogenous 43k D protein, which has been cloned and chemically characterised in man and animals (1–3). AF was originally designated as an antisecretory protein (1). However, AF also mediates a potent anti-inflammatory effect, which was clearly demonstrated by the effects of AF therapy in patients with ulcerative colitis or Crohn's disease (4–6). It is expressed in most tissues and is present in plasma, either in a free form or associated with the proteasome (7–9). In healthy individuals, most of the AF found in plasma is present in inactive form. If, however, the intestine is exposed to a diarrhoea-inducing agent such as bacterial enterotoxins, AF is rapidly transformed from *inactive* to *active* form (8). A raised concentration of *active* AF contributes to the normalisation of the intestinal transport of water and ions (10–12). Thus, AF counteracts diarrhoea irrespective of the inducing agent and functions as an essential part of the defence system against disturbances in the homoeostasis of the body.

Abbreviation
AF, Antisecretory factor.

Diarrhoeal diseases among young children are still a substantial problem in terms of morbidity and mortality in low–middle income (LMI) countries such as Pakistan (13,14). The survival of these young children requires a generous input from the healthcare systems, also focusing on the resultant malnutrition. Nearly 45% of these young children under the age of five are undernourished (14). Thus, the healthcare systems are overloaded with the considerable burden of patients with diarrhoeal diseases with both short- and long-term medical and economical effects (14,15).

Key notes

- In a randomised placebo-controlled trial, 36 children 7 to 60 months old with acute diarrhoea, were allocated to Salovum® (AF) group (high doses) or placebo group.
- An effective response i.e. stool frequency reduced to less than 3 and improved the stool consistency to normal, was obtained in 7–10 h in the Salovum® group versus 18 h in the placebo group. No side effects were demonstrated.
- AF in high doses produces an efficient and safe response in childhood diarrhoea.

The preformed active AF can be administrated as a drink prepared from spray-dried egg yolk with a high content of AF (B221®, Salovum®). The AF in egg yolk originates from hens in which AF has been induced by means of a special feed. The endogenous AF synthesis in the hen is reflected in the raised concentration of AF in the egg yolk, which is 500–1000 times higher in the Salovum than in control egg yolk (16). The preformed AF in the egg yolk is readily absorbed from the human intestine, and the diseased patient can register the antisecretory effect already 20–30 min after ingestion (6).

A previous randomised, placebo-controlled trial with Salovum was performed in 240 young Pakistani infants, aged 7–24 months, with acute or chronic diarrhoea, giving them an initial dose of 2 grams of Salovum, followed by 2 g every 5 h (17). The numbers and consistency of stools were continuously followed. The results demonstrated a reduction in stool numbers ($p = 0.0054$) and that a total recovery within 3 days was more common ($p < 0.001$) in the Salovum group when compared to the placebo group. Later, in a pilot study, we tested the response to diarrhoeal illness in a separate group of children (7–60 months) where we doubled the initial dose of Salovum (18). The patients responded with a further reduction in the number of stools and normal stool consistency, which was obtained significantly faster in the Salovum group than in the control group. No adverse effects of Salovum intake have so far been registered in any of our studies (5,6,17,18).

The current study was designed as a placebo-controlled experimental investigation of children suffering from acute diarrhoea with mild-to-moderate dehydration at the time of registration. We aimed at reaching the antidiarrhoeal effects faster by further increasing the Salovum dose during a 3-day-long treatment.

MATERIALS AND METHODS

Study setting and design

The placebo-controlled, randomised experimental study was conducted at the Children's Hospital of Institute of Child Health, Lahore, Pakistan. Salovum with a high content of active AF *versus* placebo containing a low dose (17) of active AF was given. The data were collected from April to July, 2012.

Sample size and study population

Based on our previous study, a sample size of 36 children was used, with 18 cases and 18 controls, using a 95% confidence interval and an alpha level of 0.05, with a power of 80%, to detect a difference of 28% as a successful outcome in the two groups (17,19). The target group was selected from patients reporting to the outpatient department and from inpatients in the diarrhoea unit. The children were aged 7–60 months and had reported with acute watery diarrhoea with mild-to-moderate dehydration. Both breastfed and nonbreastfed children were included. Patients with known egg allergies were excluded from the study.

The identified children were randomly allocated to either the Salovum group or the placebo group using random number tables, generating lists for the two groups. Salovum was provided in sachets of freeze-dried egg yolk and marked as Sachet A, while the placebo was sachets of spray-dried egg yolk powder marked as Sachet B. The mothers and the health team were kept blinded to which sachet they were given. Both sachets looked exactly alike, and the code stayed with the PI throughout the study period released only after the analysis was completed. The number of children included in the study was obtained after screening from 62 children reporting with different severity of acute diarrhoea. Twenty-two children were excluded based on the inclusion criteria and refused to participate in the study. Forty children were then randomised to either the Salovum or the placebo groups. Analyses were performed on 36 children, 18 from each group. See Trial Profile (Fig. 1).

The health team consisting of a paediatrician, medical students and lady health visitors was trained to record and ensure the intake of the egg yolk drinks. They were, however, blinded to the actual contents of the sachets. All of the patient parameters were recorded every 5 h during their stay in the hospital, including the number of stools passed, consistency of stools, state of hydration and weight. The time of admission, starting of feeding Salovum or placebo, reduction in stool frequency, normalisation of stool consistency and the time of discharge were noted. The discharge criteria were considered as reduction in the number of stools passed to <3 in 24 h and a normalisation of the stool consistency.

Methods

After start of admission and registration of parent's consent, the identified children received sachets according to the respective group. Age, gender, date of diarrhoea onset, frequency and consistency of stools, hydration status and weight were recorded. Four sachets á 4 g, that is, 16 grams of Salovum or placebo egg yolk, mixed in oral rehydration solution (ORS), were given orally over a period of ½–1 h with a spoon. Two sachets, that is, 8 g, were then given every 5 h in the same way, and all the parameters were recorded again until the diarrhoea stopped, for example, the number of stools became <3 per 24 h, and the consistency was back to normal. The state of hydration and weight was also followed. All the children continued with the treatment according to the standard protocols practiced in the ward.

Statistical analysis

The data were analysed using SPSS version. 20. Bivariate analysis was performed for comparing the characteristics of the two groups. A *t*-test was performed to determine the differences in the two groups. ANOVA was applied to the two groups, and the number of stools and consistency were compared. Proportions were compared using a chi-square test or a Fisher's exact test. Since we had noted the time of admission and the time of discharge, we used a Cox regression model for survival analysis. An omnibus test for

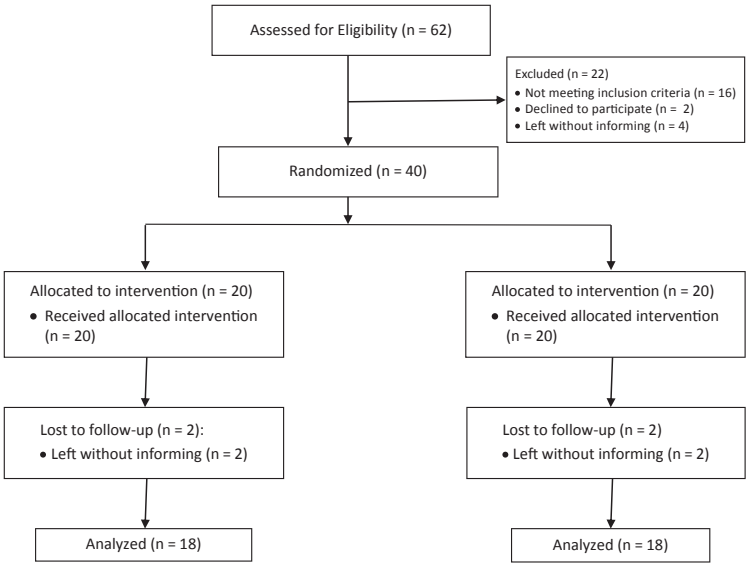

Figure 1 Trial Profile.

model coefficients was then used. Number of stools and consistency were studied in the model both separately and combined. The reliability index using Cronbach's alpha coefficient, when putting, these two variables were examined either separately or together, was high (0.83), so we could show that the curves were useful to indicate the differences in responses.

Ethical considerations

Permission to conduct the study was obtained from the Ethics Committee of the Children's Hospital. An informed consent form was prepared. Every individual had the rights to refuse participation. The consent form explained the procedures, along with the risks and benefits, although no known risks were perceived because side effects of treatment with Salovum have not been reported. The health staff read out the contents of the consent forms and clarified any apprehensions of the subjects' parents.

RESULTS

The study was completed in 18 infants in each of the Salovum and placebo groups. The age, gender, nutritional status and number of days with diarrhoea and hydration status were initially comparable in the two groups at the time of recruitment (Table 1). The Salovum and placebo groups did not differ as to age and gender. The mean number of days of diarrhoea was 2.83 ± 1.46 in the Salovum group and 2.94 ± 1.92 in the placebo group ($p = 0.8466$). However, the proportion of children with watery stools initially was significantly higher in the Salovum group when compared to the placebo group ($p = 0.043$). This indicated that the children in the experimental group were worse off. The mean and SD for weight for age did not differ significantly between the two groups.

Table 1 Characteristics of the children in the Salovum group and the placebo group at the time of recruitment

	AF group	Placebo group	p-value
Age in months			
Mean ± SD	12.33 ± 7.75	15.11 ± 12.20	0.4198
Gender			
Males	11 (61.11)	13 (72.22)	0.4936
Females	7 (38.89)	5 (27.78)	
Number of Days of diarrhea at the time of recruitment			
Mean ± SD	2.83 ± 1.46	2.94 ± 1.92	0.8466
Consistency of stools at the time of recruitment			
Loose	8 (44.44)	14 (77.78)	0.043
Watery	10 (55.56)	4 (22.22)	
Nutritional status at the time of recruitment			
Mean ± SD	8.02 ± 1.63	8.73 ± 2.24	0.4312
Hydration status			
Mild dehydration	14 (77.78)	13 (72.22)	0.5678
Moderate dehydration	4 (22.23)	5 (27.78)	

The mild and moderate dehydration status did not differ between the two groups.

The probability of 'survival' was defined as the number of stools becoming <3 in 24 h, as the basis for the 'survival' curves for the Salovum and placebo groups. Using the Cox proportional hazard model, two curves were drawn and the omnibus test was used to compare them (Fig. 2). The chi-square value was 25.206 with 1 df, $p = 0.0001$. The total

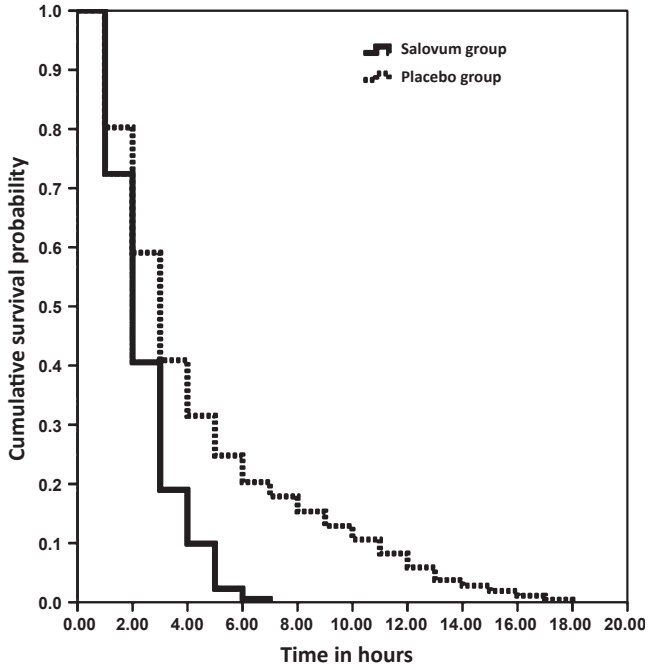

Figure 2 The reduction in the number of stools (survival) over time measured in hours in the two groups. The overall difference in the time of recovery between the Salovum group was significantly less (7 h) than the recovery time (18 h) in the placebo group (p = 0.0001).

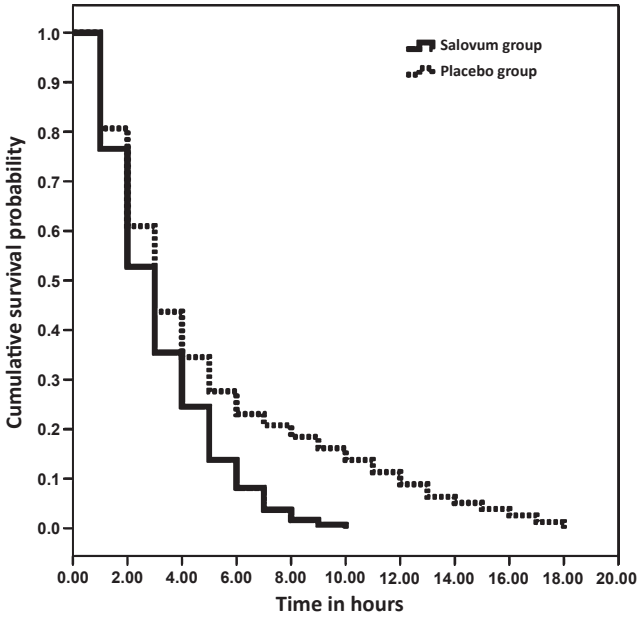

Figure 3 The return to normal stool consistency from watery and loose in the two groups over time. The difference in the proportion of children with normal stooling was reached by 10 h in the Salovum group compared with 18 h in the placebo group (p = 0.031).

duration of disease time was 7 h in the Salovum group compared with 18 h in the placebo group.

The transition of stools from watery and loose to normal is illustrated in Fig. 3. The two curves show an earlier response in the Salovum group when compared to the placebo group, that is, a total duration in the Salovum group of 10 h compared with 18 h in the placebo group. When compared, the difference in time for the events (return to normal consistency) in the two groups showed a significant difference (chi-square value = 4.681 with 1df, p = 0.031).

The frequency of stools and changes in consistency in the Salovum group and in the placebo group is shown in Fig. 4. The proportions over time are significantly different although the time taken for consistency to normalise was greater when compared separately. Thus, the overall time was 35 h for the Salovum group when compared to the 70 h in the placebo group. The data were censored at 72 h (chi-square value 10.290 with 1df, p = 0.001).

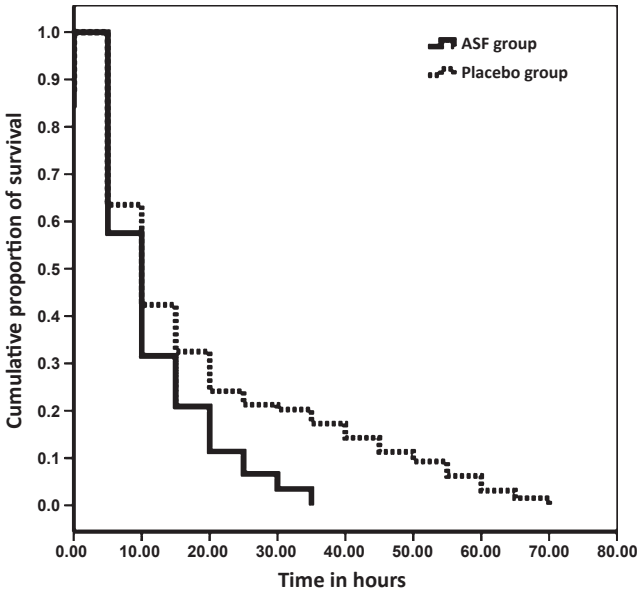

Figure 4 The reduction in number of stools and consistency returning to normal. The recovery from both the events was significantly earlier in the Salovum group (total hours = 35) when compared to the placebo group (total hours = 70, p = 0.001).

DISCUSSION

The present double-blinded, randomised and placebo-controlled study demonstrates how the endogenous protein AF efficiently counteracted childhood diarrhoea. This is in agreement with previous studies in man and animals (3–6, 20–22). The recovery from disease was achieved significantly faster in the Salovum group than in the controls, despite the fact that the AF-treated group at admission showed signs of more severe illness.

The strengths of the study were the design and that the sample size calculated was based on our previous study (17) with a known level of outcome. The team was well trained

through their previous experience from randomised studies. Through constant training and vigilant record keeping over time, we could considerably reduce observer bias. As the team was present round the clock, observations were probably not missed.

No faecal, bacteriological cultivations were performed on the patients in the present study, and the diarrhoeal aetiology in the different patients can therefore not be described. It is highly unlikely, however, that all patients were affected by the same causative agent. This agrees with the fact that AF functions as a principal regulator of intestinal water and ion transport and performs its action irrespectively of the diarrhoea-inducing mechanisms (4,20,21). The protective and regulating influence of AF is presumably mediated via its capacity to normalise secretion and possibly also via its anti-inflammatory capacity. The wide range of biological capability probably reflects that AF binds with high affinity to flotillin-1 in the cellular membrane (22). The flottilin-1 cluster on the cellular membrane is rich in lipids and receptors and functions as a controlling authority concerning the transport capability of water and ions across the membrane.

We propose that AF functions as part of the innate and not of the adaptive immune response. Due to the variety of functions, we tentatively suggest that AF is capable of modulating inducible NO synthase and to significantly regulate certain complement factors, especially C3c and factor H (23). Ongoing work will provide more information about the actions of AF in the damaged tissue.

The increase in initial 16-g dose of Salovum and the following 8 grams every 5 h reduced the numbers of stools to <3 in 24 h, and the patients reached normal consistency within 10 h. This is far faster than in our previous study using lower doses, where the mean frequency of stools/day had declined to 4.8 after 3 days. Thus, by increasing both the initial and subsequent doses of Salovum, both the reduction in stool numbers and normalisation of stool consistency were reached significantly faster than in previous studies. These results tentatively suggest that a further increase in Salovum dosage and reduction in time between the doses will be even more optimal for reaching normalisation without any side effects. Hence, an initial dose of 16 g followed by 8 g every 3 h would probably lead to a faster normalisation of the intestinal water and ion transport. It is of importance to counteract diarrhoea as fast as possible to restore the body water and ion balance. The faster this clinical goal is achieved, the less is the risk for the diarrhoea to develop further into a chronic or fatal diarrhoeal disease (14,15).

Age, gender, nutritional, hydration status and number of days with diarrhoeal disease did not differ between the two study groups at the time of patient recruitment. Furthermore, intake of fluid and nutrients during the hospital stay did not differ between the Salovum and placebo groups. The proportions of patients with watery diarrhoea, when included, was, however, significantly larger in the Salovum group than in the placebo group. This lack of equality between the groups did not seem to influence the positive effect of Salovum on the clinical outcome of the AF treatment. In a previous study performed on 20 severely dehydrated, adult cholera patients, no effects of Salovum treatment were observed during the first 24 h of surveillance (24). The Salovum dose used during this time was a total of about 1 g per kg body weight administered every 2 h; thereafter, 0.5 g per kg body weight per 24 h was given. In the present study with mild-to-moderate dehydrated children, a total of 7 g of Salovum per kg body weight was administered every 5 h during the first 24 h of surveillance; thereafter, 6 g per kg body weight per 24 h was given. The lack of Salovum effect in the adult study might consequently be explained by an insufficient dose of Salovum used.

Salovum consists of spray-dried egg yolk only and is therefore classified by the EU authorities as a 'food for medical purposes'. This fact makes it easy to combine Salovum with other forms of medical treatment. Another advantage of Salovum treatment is that no adverse reactions have so far been registered, which provided that patients with known egg allergy are excluded. Salovum might also be administered to severely ill patients via feeding by a nasogastric tube or by colonic absorption provided via a Salovum enema. A more longstanding and continuous stimulation of the endogenous AF system can be achieved by feeding the children with SPC-Flakes, either as finely grinded and mixed with milk or ordinary food, or as a muesli (3,4). Induction of increased AF production achieved via intake of SPC-Flakes over time might reduce the risk for infants and children in impoverished areas to the threat of repeated diarrhoeal diseases of varied aetiology.

CONFLICT OF INTEREST

None declared.

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