ARTÍCULOS ORIGINALES

Enemas de factor de crecimiento epidérmico para inducir la remisión de la colitis ulcerosa izquierda

Epidermal growth factor enemas for induction of remission in left-sided ulcerative colitis

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ABSTRACT

Introduction: Ulcerative colitis is a little known chronic inflammatory disease in colonic mucosa. The positive effect of epidermal growth factor was shown in a previous report, with enema use for treatment of mild to moderate left-sided manifestation of the disease. This evidence provided the basis for evaluating the efficacy and safety profile of a viscous solution of this product.
Methods: Thirty-one patients were randomized to three groups for daily medications during 14 days. Twelve received one 10 mg enema of epidermal growth factor dissolved in 100 mL of viscous solution whereas nine were treated with placebo enema; both groups also received 1.2 g of oral mesalamine per day. The other group included ten patients with 3 g / 100 mL of mesalamine enema. Primary end point was clinical responses after two weeks of treatment, defined as a decreased of, at least three points from baseline, the Disease Activity Index and endoscopic or histological evidences of improvement.
Results: Remission of disease was observed in all patients in the epidermal growth factor group, and six in both, mesalamine enema and placebo group. All the comparisons between groups showed statistically significant superiority for epidermal growth factor, the only product with significant reduction in disease activity index as

well as the presence and intensity of digestive symptoms in patients after treatment. None adverse event was reported.
Conclusions: The results agree with previous molecular and clinical evidences, indicating that the epidermal growth factor is effective to reduce disease activity and to induce remission. A new study involving more patients should be conducted to confirm the efficacy of the epidermal growth factor enemas.

Key words: Ulcerative colitis; Epidermal Growth Factor; Mesalamine; Clinical trial.

RESUMEN

Introducción. La colitis ulcerosa es una enfermedad inflamatoria crónica de etiología poco conocida, que afecta la mucosa del colon. El efecto positivo del factor de crecimiento epidérmico fue reportado en estudio previo con uso de enema para tratamiento de la manifestación izquierda leve o moderada de la enfermedad. Este antecedente sirvió de base para evaluar la eficacia y perfil de seguridad de una solución viscosa del producto.
Métodos. Fueron aleatorizados 31 pacientes hacia tres grupos de tratamiento diario durante 14 días. Doce recibieron enemas de 10 mg de factor de crecimiento epidérmico en 100 mL de solución viscosa, mientras nueve fueron tratados con enemas placebo conteniendo solamente solución viscosa. Ambos grupos recibieron además 1,2 g diarios de mesalacina oral. El tercer grupo incluyó 10 pacientes con mesalacina en enemas de 3g / 100 mL. La variable principal de eficacia fue la respuesta clínica al finalizar las dos semanas de tratamiento, definida como la disminución de, al menos tres puntos, el índice basal de actividad de la enfermedad acompañada de mejoría endoscópica o histológica.
Resultados. Se alcanzó remisión de la enfermedad en todos los pacientes que recibieron factor de crecimiento epidérmico y en seis de los grupos mesalacina enema y placebo. Todas las comparaciones entre grupos mostraron superioridad estadísticamente significativa para el factor de crecimiento epidérmico, único producto que logró la reducción significativa del índice de actividad de la enfermedad y de la presencia e intensidad de los síntomas digestivos en los pacientes luego del tratamiento. Ningún evento adverso fue reportado.
Conclusiones. Estos resultados son consistentes con las anteriores evidencias moleculares y clínicas que señalan al factor de crecimiento epidérmico capaz de reducir la actividad e inducir la remisión de la colitis ulcerosa. Debe ser realizado un nuevo estudio en un número mayor de pacientes para confirmar la eficacia de los enemas de factor de crecimiento epidérmico.

Palabras clave: colitis ulcerosa; Factor de crecimiento epidérmico, Mesalacina, Ensayo clínico.

INTRODUCTION

Inflammatory bowel disease is a group of disorders of unknown etiology. Its origin is attributed to a distortion in the mechanism responsible for maintaining delicately balanced the intestinal mucosa in a quiescent state of inflammation. [1] The loss of this

balance leads to a chronic debilitating disease that affects millions of people worldwide as ulcerative colitis (UC).[2] The management of UC represents a challenge for physicians, basically with regard to the control of active inflammation and process of healing in the mucosa. [3] The chronic inflammatory conditions in UC are characterized by periods of clinical exacerbation and remission.[4, 5]

A variety of therapeutic agents have been used in UC. [6] The standard therapy with aminosalicylates (5-ASA) offers only up to 80% of response. Drug as prednisone causes resistance and dependence, while others like azathioprine have serious side effects. Therefore, new therapeutic approaches are needed. [7]

Preliminary studies in human suggest that topical administration of Epidermal Growth Factor (EGF) enhances healing of skin wounds, and the systemic use is beneficial for necrotizing enterocolitis in neonates. More recent is the report about the benefit of the intralesional application of this peptide in diabetic food ulcer.[8] Specifically EGF formulated in viscous solutions was effective for induce the remission of radiation proctitis, including a significant decrease in rectal bleeding and tenesmus associated. [9] Also in patients with duodenal ulcers, the oral EGF achieved similar results compared with conventional anti-ulcer drugs.[10, 11]

A previous study conducted in England with the application of EGF enema formulated in degraded and modified gelatin carrier solution, resulted in effective treatment for active left-sided ulcerative colitis.[12] The present study was designed to examine the efficacy and security profile of EGF in viscous solution by enema for patient with mild to moderate active left-sided ulcerative colitis.

METHODS

The trial was exploratory and designed randomized, double blind and controlled by placebo and conventional treatment groups. Was approval by ethic committee of Hermanos Ameijeiras Hospital in Havana, Cuba and conducted from February 2006 to June 2008 according to the ethical principles contained in the Helsinki Declaration and following the Good Clinical Practices. The participation of all subjects was totally voluntary and this was expressed through the signing of written informed consent prior to inclusion.

Subjects were eligible if they had established diagnosis of UC, verified by the digestive symptoms, colonoscopy and histopathology. The inclusion criteria were at least 18 years of age, mild-to-moderate active left-sided disease, as defined by score of 6-10 on the Diseases Activity Index (DAI), with a proctosigmoidoscopy and histological scores £ 2. The DAI is a combination of rating scales for stool frequency, rectal bleeding, endoscopic activity and physician's global assessment (each 0-3 for no activity-severe disease). The maximum total DAI score is 12 for severe disease. Exclusion criteria were severe UC, confirmed amebiasis, pregnancy, and previous immunosuppressive or corticosteroids therapy within three months and thirty days respectively. Patients were also no considered if they had history of cancer (other than basal cell carcinoma or cervix carcinoma) as well as any other serious no compensated medical conditions.

Included patients were assigned to three groups, according to a random computer list. Treatment was applied during 14 consecutive days in hospitalized regimen, receiving mesalamine as active treatment and supplementary low waste diet as complement. Study group (I) received an EGF enema (100 mL) and 1.2 g per day of

oral formulation of mesalamine (Chron-ASA5 400 mg, Sigma Pharma). The only difference with the control group (III) was that the used a placebo (Pb) enema. The second group (II), without blinding, was treated with a mesalamine enema (Chron-ASA5 3g/100 mL, Sigma Pharma). Once finished treatment, was established a follow-up period of 12 weeks, without medications for those patients in remission or with favorable response.

Patients were evaluated by clinical, sigmoidoscopic and histological criteria before start and after conclude the treatment (week 2). Also evaluated at week 6 and 14 as follow-up for long-time response. The clinical disease activity was determinate by DAI. Also, patients completed daily a digestive and extra intestinal symptoms questionnaire during treatment period. In case of relapse, was given conventional treatment to patient. The original blinding level was conserved throughout the study period.

The EGF enema was indistinguishable from enema placebo and consisted of 10 mg of peptide in 100 mL of viscous solution, while placebo contains only viscous carrier. The selected doses of EGF was recommended by Playford after restitution response in intestinal cell lines and was later confirmed by the results of a clinical study very similar to ours, carried out in England[12]. In our study, the dose was doubled due to enhance demonstrated dose-dependent effect of EGF on the healing and because we had successful clinical experiences with 10 mg in previous trials performed for other diseases[9-11]. The period of medications was taken identical to Playford's study, because had promising results with this design.

Enemas were administered by assistant nursing, using a rectal catheter, before going to be at night. Patients were instructed to stay in the left lateral decubitus for retain the preparations as long as possible and then lay on each side for 15 minutes to ensure maximal contact of the enema preparation with mucosa.

Sigmoendoscopic procedure was performed using standard equipment. Before inclusion was necessary a complete colonoscopy, but subsequent were limited to the distal extension. Each examination was coded (0= normal, 1= mild, 2= moderate, 3= severe) according to the appearance of the mucosa. Mild grade was defined by erythema, decreased vascular pattern, friability of mucosa, and single ulcer lesions; additional absent vascular pattern, multiple ulcer lesions and erosions characterized the moderate grade and the presence of spontaneous bleeding, ulcerous lesions and lumen narrowing categorized as severe grade. During colonoscopy three biopsies were taken from different portions of the inflamed mucosa to be analyzed blinded by histopathology and classified as normal, mild, moderate o severe activity.

Primary end point was clinical treatment responses at two weeks, defined as a decreased of at least 3 points from baseline DAI and endoscopic or histological evidences of improvement. This global variable was expressed in four categories, including remission as principal. Each individual evaluated variable was a secondary treatment end point. Occurrences of disease activity relapse and relapse-free interval were used as long-time efficacy variables at 6 and 14 weeks.

Analyses were performed according to the intention-to-treat principle and using Bayesian statistics as an alternative to the impossibility of conventional methods in the small number of sample in each group. For the purpose of this study, the Bayes Factor evidence a tendency in favour of the hypothesis of dependency, becoming more evident as its value is greater than 1.0.

RESULTS

Thirty-one patients were included. Twelve were randomized to receive EGF enema and nine to be treated with placebo enema, the ten remaining were assigned to mesalamine enema group. Baseline demographics of the study population (table 1) showed that male : female ratio was 9 : 22, with the majority (87.1 %) of patients white and 42.0 % older than fifty years of age. No patients were receiving topical u oral mesalamine therapy at time of recruitment.

Table 1. Demographic information of patients

Variables		EGF+ASA-5	ASA-5	Pb+ASA-5	Total
N		12	10	9	31
Gender	Male	4 (33.3%)	3 (30.0%)	2 (22.2%)	9 (29.0%)
	Female	8 (66.7%)	7 (70.0%)	7 (77.8%)	22 (71.0%)
Race	White	10 (83.3%)	8 (80.0%)	9 (100.0%)	27 (87.1%)
	No white	2 (16.6%)	2 (20.0%)	0	4 (12.9%)
Age	Median ± QR	54.0 ± 10.2	55.5 ± 12.8	51.0 ± 24.5	55.0 ± 14.0
	Range	24; 64	41; 65	35; 65	24; 65

Data source: case report file

The initial classification of the disease based on the affected area, was primarily completed left-sided colitis in 24 patients (77.4 %), rectosigmoid 5 (16.1 %) and 2 (6.5 %) ulcerative lesions in the rectal mucosa. Before the start of treatment were gastrointestinal symptoms in 29 patients (93.5 %) and extra intestinal in 7 (22.6 %). The presence of mucus and pus in stool along with the occurrence of tenesmus associated to abdominal pain were the most common gastrointestinal manifestation. The median DAI score at the beginning was 5, with 17 patients (54.8 %) presented moderate endoscopic inflammation and histological activity of disease.

During the course of the study, was not necessary to discontinue treatment in any patient. We obtained maximum compliance of the protocol in all subjects included. At the end of the two weeks of treatment, all patients in the EGF group had clinical response evaluated as remission (table 2), as compared with 6 of 10 (60.0 %) and 6 of 9 (66.7 %) in the mesalamine enema and placebo group respectively. The estimate of the Bayesian confidence intervals of 90 and 95% concluded statistical significant superiority for the remission in group of EGF enema, because they showed no points of intersection with the two other similar groups.

Table 2. End point efficacy evaluation

Variables	EGF+ASA-5	ASA-5	Pb+ASA-5	Total	Bayes Factor
N	12	10	9	31	
Remission	12 (100.0%)	6 (60.0%)	6 (66.7%)	24 (77.4%)	General: 6.681 EGF vs. ASA-5: 8.0 EGF vs. Pb: 3.8
IC 95%	(92.0; 100.0)	(30.0; 86.0)	(34.9; 91.1)	(61.3; 89.9)	
IC 90%	(95.1, 100.0)	(34.5; 82.8)	(39.9; 88.5)	(64.1; 88.3)	
Favorable response	0	1 (10.0%)	0	1 (3.2%)	
No response	0	2 (20.0%)	3 (33.3%)	5 (16.1%)	
Progression	0	1 (10.0%)	0	1 (3.2%)	

Data source: case report file

There are no detectable differences between groups in the initial or final DAI score. However, although three showed a reduction in the previous score, only the group treated with epidermal growth factor, achieved a statistically significant decrease (table 3). Clinically, the periodic evolution of digestive symptoms led to the identification much more rapid improvement in the mucosa of those treated with EGF (see figure 1). This finding was confirmed by the disappearance of ulcerous lesions and mucus observed through endoscopies, as well as, enhanced by higher number of patients receiving EGF with reduction in histological score.

Table 3. Score variation for secondary variable

Variables		EGF+ ASA-5	ASA-5	Pb + ASA-5
N		12	10	9
Diseases Activity Index (mean)	Week 0	5.9	6.0	5.7
	Week 2	1.2	2.2	2.1
Confident interval 95% (bayesian)		(-0.3; 2.3)	(-0.6; 3.6)	(-4.1; 4.1)
Sigmoidoscopic Index (mean)	Week 0	1.58	1.60	1.44
	Week 2	0.33	0.60	0.56
Confident interval 95% (bayesian)		(39.0; 88.8)	(30.0; 86.0)	(34.9; 91.1)
Histological Index (mean)	Week 0	1.67	1.50	1.44
	Week 2	0.50	0.70	0.78
Confident interval 95% (bayesian)		(58.2; 97.5)	(65.3; 99.6)	(34.9; 91.1)

Data source: case report file
DAI scale: normal (0-3), mild (3-6), moderate (7-10), severe (>10)
Sigmoidoscopic and histological scale: normal (0), mild (1), moderate (2), severe (3)

Our observations indicate that present between 3-4 stools above normal, mucus in stool, spontaneous rectal bleeding and exudations from ulcers in the mucosa can be negative prognostic factors for a favorable outcome to treatment.

The satisfactory results achieved with the treatment, continued to follow up. Only 5 patients (2 EGF and placebo and 1 mesalamine) had relapse due to worsening of symptoms and increase in endoscopic or histological disease activity. Since almost all patients did not undergo follow-up sigmoidoscopy, we defined remission based on clinical criteria consisting of a normal daily stool frequency and no blood in stool. None patients required corticosteroid treatment and relapse-free interval for patients treated with EGF was 107 days, without significant differences from placebo or mesalamine.

Those features identified as probable negative prognostic factors for response to therapy, repeated in the individuals who lost remission. The most important elements in relapse were the recurrence of chronic lesions characterized by rectosigmoid mucosal ulceration and an increased number of inflammatory cells in the lamina including polymorph nuclear cells infiltration.

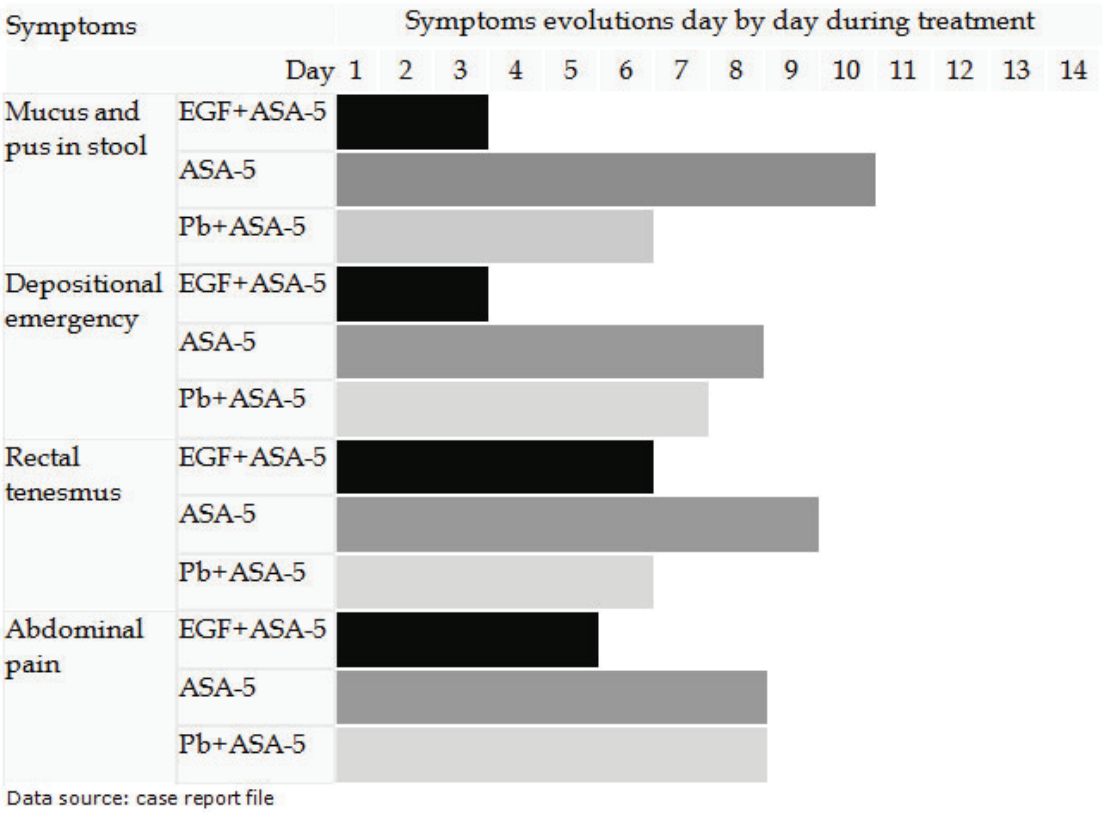

Fig. 1. Statistical significant of efficacy response.

DISCUSSION

The baseline patients characteristic and disease parameters of the patients were in complete match with the typical profile of the patients with UC[13, 14] and in our specifically trial, the demographic finding are in corresponding with the distributions for gender and race described for Cuban populations.

Our results showed statistically significant efficacy for the EGF enema administered daily in combination with oral mesalamine. The favorable evidences with this product were obtained under controlled conditions with placebo and the best available active treatment. The improvement in condition of all patients who received EGF until to achieve remission of the disease in two weeks of treatment was a corroboration of the outcomes obtained by Playford[12] in a similar study made in England. Although in our case, exceed 17.0% in number of remission.

The bigger efficiency achieved in the study of Cuba was attributed first, a dose increased to 10 mg of EGF, which enhanced the protector effect on gastrointestinal mucosa cells, very often described as a dose-dependent mechanism. Second the use of EGF in a viscous carrier solution formulation with glycerin, parabens and carboxymethyl cellulose as principal component, polymer with potential anti-inflammatory contributions and causes a pleasant feeling of freshness and comfort when the product are on the affected mucosa. Precisely, these beneficial effects of the viscous formulation used were considered among the reasons for the high percentage of patients with placebo and remission of the disease, even better to mesalamine enema. Both, hospitalized regimen and supplementary diet were two additional elements that contributed to a successful evolution during treatment.

The result achieved for the EGF according to global efficacy variable was maintained for the clinical evaluation of individual patients with a statistically significant reduction in scores on the DAI, confirming the active participation of this peptide in re-establishing gastrointestinal cells continuity.[15, 16] Furthermore, the early improvement in the digestive symptoms achieved with the EGF is consistent with the expected and necessary defensive and restorative actions on the colonic mucosa.[17, 18]

The therapeutic management of ulcerative colitis includes a maintenance treatment following the induction of remission.[19] In our exploratory study, the significant reduction of disease activity and remission induced by EGF could not be reached with the same magnitude in the follow-up assessments compared with placebo or mesalamine enema. This result suggests that EGF should be not considered as an alternative for maintenance period, the same suggestion was made by Playford. In both cases, was considered the potential effect of EGF on cell proliferation and stimulation of premalignant lesions.

Rectal administration has recognized advantages to ensure the delivery of quantities of product required intact for optimal results on left-side UC.[20] In our study, also for Playford, the application of EGF in enema was the guarantee for the results described, without the occurrence of adverse events for our patients despite the use of double dose.

In summary, our findings confirm that the EGF contributes to the immediate repair of the epithelial integrity and the preservation of metabolic homeostasis of the luminal and extra luminal structures. Facilitation of spontaneous bleeding disappearance and healing of ulcers in mucosa were two clinical benefits associated with the use of the product. Further studies are warranted to confirm optimal doses and extend these finding to more extension of UC, and to define the potential benefits offered by EGF directly compared with high doses mesalamine or as an alternative to glucocorticoids.

Acknowledgements

The authors thank Heber Biotec S.A. for the supply of the investigational product. The Ministry of Public Health of Cuba supported the work. Authors H.N.C, C.M.V.S, O.C.L.D, C.H.B.B, G.M.S and P.A.L.S. are employees of the Centre for Biological Research, which is part of the Center for Genetic Engineering and Biotechnology (CIGB), Havana network, where viscous solution of EGF is produced. Authors R.P.M and J.B.A are also employees of CIGB, working in the Division of Development and Biomedical Research respectively. The rest of the authors have no conflict of interests.

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Recibido: 4 de julio de 2012.
Aprobado: 26 de septiembre de 2012.

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