RESEARCH

Initial evidence of safety and clinical effect of recombinant streptokinase suppository in acute hemorrhoidal disease. Open, proof-of-concept, pilot trial

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ABSTRACT

A proof-of-concept, pilot clinical trial was carried out in 2 hospitals, to evaluate the safety of recombinant streptokinase (rSK) administered by the rectal route in patients with acute hemorrhoidal disease (AHD). Suppositories containing 200 000 IU rSK were given every 6 hours, up to 4 applications. The patients , after discharge, were seen daily in follow-up visits up to10 days. Symptoms, lesion size, edema and inflammation were evaluated. Ten patients were included. The rSK suppository was safe and tolerable. The adverse events reported were minimal (only ardor and anal itching in only one patient), both with minor intensity which did not require treatment, and with low causality relationship of the product since they could be explained by the underlying disease as well. Symptoms disappeared at 24 hours in 7 patients. Complete recovery was achieved in most of the patients (90%) in 5 days. Only one patient needed surgical thrombectomy. rSK suppositories are safe and showed initial efficacy data. It could become a new therapeutic option for hemorrhoidal crisis if results are confirmed in further and controlled studies.

Keywords: streptokinase, thrombolysis, suppository, hemorrhoidal crisis

Biotecnología Aplicada 2010;27:277-280

RESUMEN

Evidencia inicial de la seguridad y el efecto clínico del supositorio de estreptoquinasa recombinante en la enfermedad hemorroidal aguda. Estudio piloto, abierto, de prueba de concepto. Se realizó un ensayo clínico piloto, prueba de concepto en 2 hospitales, con el objetivo primario de evaluar la seguridad de la estreptoquinasa recombinante administrada por vía rectal en pacientes con enfermedad hemorroidal aguda. Se administraron 4 supositorios de 200 000 IU de rSK distribuidos cada 6 horas. Los pacientes, tras el alta, fueron vistos a diario (en las visitas de seguimiento) hasta 10 días. Se evaluaron los síntomas, el tamaño de la lesión, el edema y la inflamación. Diez pacientes fueron incluidos. El supositorio de rSK fue seguro y tolerable. Fueron mínimos los eventos adversos reportados (sólo ardor y prurito anal en un solo paciente), ambos de intensidad leve, que cedieron espontáneamente y con baja relación de causalidad con el producto objeto de estudio. Los síntomas desaparecieron a las 24 horas en 7 pacientes. Se obtuvo respuesta completa en la mayoría de los pacientes (90%) a los 5 días. Sólo un paciente requirió trombectomía. Los supositorios de rSK son seguros y muestran datos iniciales de eficacia. Podría convertirse en una nueva opción terapéutica para la crisis hemorroidal si los resultados se confirman en sucesivos estudios, controlados, en un mayor números de pacientes.

Palabras clave: estreptoquinasa, trombolisis, supositorio, crisis hemorroidal

*I*ntroduction

Although the exact incidence of hemorrhoidal disease is unknown, 10 to 25% of the adult population is thought to be affected. Symptoms seem to be more common in older individuals, with a prevalence peak at 45 to 65 years [1]. Studies evaluating the epidemiology of hemorrhoids showed that 10 million people in the United States reported hemorrhoids, for a prevalence of 4.4%. In both genders, a peak is noted between 45 and 65 years of age; development of hemorrhoids before the age of 20 is unusual, and Caucasians

1. Nelson RL, Abcarian H, Davis FG, Persky V. Prevalence of benign anorectal disease in a randomly selected population. Dis Colon Rectum 1995;38:341.

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are affected more frequently than African-Americans [2, 3].

The initial treatment of the hemorrhoidal illness consists of general conservative measures (hygienic-dietetic, life style changes, symptomatic treatment) directed mainly to restore the intestinal habit and to diminish the local symptoms. Although several medicines have been tested for the specific treatment, significant benefits have not been obtained to control this condition [4-8]. Therefore, in an important group of patients, the surgical procedure becomes the final solution [9]. Management of the hemorrhoidal crisis depends on the intensity of the signs and symptoms and can change in patients with thrombosis, important prolapse or profuse hemorrhage. In such cases, the surgical solution is indicated (hemorrhoidectomy, thrombectomy, ligation, sclerotherapy, infrared photoclotting, cryo and laser therapy) [9-11].

Streptokinase (SK) is an indirect fibrinolytic drug that interacts with plasminogen, forming an active complex with protease action that activates plasminogen into plasmin. The efficacy of SK to reduce the mortality in the acute myocardial infarction has been demonstrated in large clinical trials [12-15] and is currently used for that purpose [16, 17] as well as, other thrombotic diseases [18, 19].

Venous thrombosis have been also treated successfully with thrombolytics [20-23]. At the same time, an anti-inflammatory action has been reported for SK, based on lysis of microthrombi, present at the inflammation site. Therefore, the local application of SK on acute hemorrhoid episodes, where inflammation and eventually thrombosis are present, seemed rational. The concept was first tested, in an experimental model for hemorrhoidal disease in rabbits, where illness resolution was evidenced and no SK could be detected in the general circulation (unpublished results). The purpose of this work was to evaluate the safety of this procedure in patients with acute hemorrhoidal disease. The first evidences of efficacy are also reported.

Materials and methods

A pilot clinical trial was carried out in 2 hospitals. Patients older than 18 years-old presenting with acute hemorrhoidal illness, who gave their written informed consent to participate were eligible. Exclusion criteria were SK administration in the previous 6 months, antecedents of intracranial hemorrhage, allergy to SK or salicylates, stroke, intracranial surgery or skull trauma less than 3 months before and any other condition where there is risk of bleeding or it would be difficult to handle because of its location. Patients with hemorrhoidal disease caused by portal hypertension, with septic or active hemorrhagic complications or associated with abscesses, fistula or cancer, were also excluded. The protocol followed the Declaration of Helsinki guidelines and was approved by the Ethics Committees of the participating hospitals and by the Cuban Regulatory Authority.

Recombinant streptokinase was produced in *Escherichia coli* at the Center for Genetic Engineering and Biotechnology, Havana [24]. Suppositories were prepared containing 200 000 IU of rSK, thimerosal, sorbitan monostearate (Span 60), sodium salicylate, and hard fat (Witepsol W25). All patients received them by the rectal route, one every 6 hours up to 4 suppositories. The patients were hospitalized during the first 24 hours and were evaluated 48 hours after discharge and at 10 days follow-up. Concomitant treatment included high-fiber diet, abundant liquid ingestion, rest in *decubitus* position; local hygiene, sitz bath with warm water 3 times per day, and oral analgesics if pain.

The aim of the trial was to evaluate the safety of the product, since it was the first-in-man use, so the type, duration, severity, outcome, and causality relationship of the adverse events were carefully registered. A qualitative assessment was used to classify the causal relationship as definite, probable, possible or doubtful [25]. The severity of the adverse events was classified upon three levels: (a) mild, if no therapy was necessary, (b) moderate, if specific treatment was needed, and (c) severe, when hospitalization or its prolongation was required, and if the reaction was life-threatening or contributed to patient's death.

For this purpose, patients were evaluated through interview and physical examination, both general and local, upon arrival, at 24, 48 hours and 10 days post-discharge and at any other moment when adverse events appeared. Adverse reactions known for SK (fever, shakes, tremors, nausea, vomiting, low blood pressure, hemorrhages and allergy), were specially searched. Hemostasis (thrombin time, fibrinogen, and fibrinogen degradation products (FDP)) was checked at 0, 24 and 48 h. The presence of anti-streptokinase antibodies was determined at the beginning and 10 days after discharge using a sandwich type ELISA method previously described [26].

Clinical effect on the illness was evaluated according to pain intensity (measured with a 10-level analogue visual scale), edema (presence or not), and lesion size (larger and smaller diameters). The clinical response was assessed at 48 hours according to the following criteria: a) Complete response: disappearance of pain and edema, and lesion size reduction; b) Partial response: two-level reduction of pain intensity or disappearance of edema, or lesion size reduction; c) No response: if no changes or any worsening occurred. Healing was considered during the 10-day follow-up. A therapeutic failure was given by no response, treatment interruptions, need for any surgical procedure, or not healing at 10 days post-release. The confidence intervals and the probabilities of cure and complete response were estimated using a Bayesian logistic model for fixed effects in WinBUGS14 package.

Results

Ten patients were included, all completed the treatment schedule and 10-day follow-up. Table 1 shows the characteristics of the patients. Most were male (80%), white (80%), 32 to 49 years old, from 1 to 11 days ill. All patients had external hemorrhoids (in one also internal, grade III); 8 of them had anal pain (4 moderate); 8 anal edema; lesion diameters ranged between 0.6 and 3.0 cm. Only 2 patients referred the use of analgesics.

The product was well tolerated. Severe or unexpected adverse events were not reported and there were no withdrawals for this cause. Only 2 adverse events were registered in the same patient. Those were ardor

2. Hulme-Moir M, Bartolo DC. Hemorrhoids. Gastroenterol Clin North Am 2001; 30:183-97.

3. Johanson JF, Sonnenberg A. The prevalence of hemorrhoids and chronic constipation. An epidemiologic study. Gastroenterology 1990;98:380-6.

4. Alonso-Coello P, Castillejo MM. Office evaluation and treatment of hemorrhoids. J Fam Pract 2003;52(5):366-74.

5. Godeberge P. Daflon 500 mg in the treatment of hemorrhoidal disease: a demonstrated efficacy in comparison with placebo. Angiology 1994;45:574-8.

6. Cospite M. Double-bind, placebo controlled evaluation of shemical activity and Safety of Daflon 500 rng in the treatment of acute hemorrhoids. Angiology 1994; 45(2):566-73.

7. Misra MC, Parshad R. Randomized clinical trial of micronized flavonoids in the early control of bleeding from acute internal haemorrhoids. Br J Surg 2000;87: 868-72.

8. Mentes BB, Gorgul A, Tatlicioglu E, Ayoglu F, Unal S. Efficacy of calcium dobesilate in treating acute attacks of hemorrhoidal disease. Dis Colon Rectum 2001; 44:1489-95.

9. De Miguel M, Oteiza F, Ciga MA, Ortiz H. Tratamiento quirúrgico de las hemorroides. Cir Esp 2005;78 Suppl 3:15-23.

10. Hull T. Examination and Diseases of the Anorectum. In: Feldman M, Tschumy WO, Friedman LS, Sleisenger MH, editores. Sleisenger & Fordtran's Gastrointestinal and Liver Disease, 7th ed. Saunders: Philadelphia, 2002, p. 2277-93.

11. Martinessi VM. Patología hemorroidal. Enfoque terapéutico integral: lugar de la cirugía ambulatoria. Trabajo de tesis; Universidad Nacional de Córdoba. Facultad de Ciencias Médicas, Argentina, 2006.

12. Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI). Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Lancet 1986;1:397-401.

13. The GUSTO Investigators. An International randomized trial comparing four thrombolytic strategies for acute myocardial infarction. N Engl J Med 1993;329: 673-82

14. Gruppo Italiano per lo Studio della Soppravvivenza nell' Infarto Miocardico (GISSI-2). A factorial randomised trial of Alteplase vs. Streptokinase and heparin versus no heparin among 12490 patients with acute myocardial infarction. Lancet 1990;336:65-71.

15. ISIS-3 (Third International Study of Infarct Survival). Collaborative Group. ISIS-3: A randomized comparison of streptokinase vs tissue plasminogen activator vs anistreplase and of aspirin plus heparin vs aspirin alone among 41299 cases of suspected acute myocardial infarction. Lancet 1992;339:753-70.

16. The TERIMA Group of Investigator. TERIMA-2: national extension of thrombolytic treatment with recombinant streptokinase in acute myocardial infarct in Cuba. Thromb Haemost 2000;84(6):949-54.

Table 1. Patient characteristics and clinical evaluations

Characteristic	1	2	3	4	5	6	7	8	9	10
Gender	Male	Male	Male	Female	Male	Female	Female	Male	Male	Female
Ethnicity	Black	White	Mestizo	White	White	White	White	White	White	White
Age (years)	34	45	37	36	32	34	33	34	49	48
Type of hemorrhoids	External / Inner	External	External	External	External	External	External	External	External	External
Crisis duration (days)	10	10	8	3	3	7	5	7	4	4
Anal pain (intensity)	Moderate	2 x 2	Moderate	Mild	Mild	No pain	Moderate	Mild	Mild	No pain
Lesion diameter (cm) (Horizontal x Vertical)	2 x 1	2 x 2	3 x 2	1 x 1.5	1 x 1	1 x 1	1 x 2	3 x 3	1.3 x 3	0.6 x 1.5
Initial symptoms[a]	AD, AE, CO, MF, TE	AD, CO, MF	AD, AE, TE	AD, AE, AP, TE, RB	AD	AD, AE, AP, CO, TE	AE, AP, BU, CO, TE	AE, AP, BU, CO, MF, TE	AD, AE	AD, AE, MF
24 hours evaluation	AD, CO, MF	-	-	-	-	-	AP	BU	-	-
48 hours evaluation	MF	-	-	-	-	-	-	-	-	-
10 days evaluation	-	-	-	-	AP	-	-	-	-	-

[a]AD: anal discomfort; AE: anal edema; AP: anal pruritus; BU: burning sensation; CO: constipation; MF: mass feeling; RB: Rectal bleeding; TE: tenesmus.

(24 and 48 hours after the treatment) and anal pruritus (10 days after the hospital release), both mild. Their causal relationship with the product under evaluation was doubtful, since both symptoms could be also explained by the underlying illness. There were no alterations of the hemostasis parameters (thrombin time, fibrinogen, and FDP) measured. Anti-SK antibodies titers did not increase significantly with respect to basal values, 10 days after treatment (results not shown).

Clinical evolution is also shown in Table 1. The main initial signs and symptoms were anal discomfort (80%), tenesmus (60%), constipation (50%), anal pruritus (40%), and mass feeling (40%). Ardor and bleeding were present in two and one patient, respectively. At 24 hours after treatment onset, most symptoms had already disappeared. All patients had achieved a response at 48 hours after the beginning of treatment (6 complete), and 9 had healed at the 10-days follow-up. Only one patient (No. 1), who had partial response needed thrombectomy. The estimated probability of complete response at 48 hours was 0.58 ± 0.14 and the probability of healing was 0.83 ± 0.10. Median time to healing was 5 days.

Discussion

The study treatment (streptokinase suppository) was administered in all cases during the first 24 hours (1 suppository every 6 hours). In terms of safety, which was the main purpose of the trial, the results indicated that the recombinant streptokinase suppository is safe and tolerable. The adverse events reported were minimal (only ardor and anal itching in only one patient), both mild, resolved spontaneously and with low causality relation with the product. Hemostasis was not altered either, probably due to scarce systemic absorption of SK since the fibrinolytic action could be exerted after local activation in the hemorrhoidal plexus, directly on the thrombi. The total SK dose administered (800 000 IU) at 24 hours is much less hemostasis disturbing than the one used for other indications such as acute myocardial infarction (1 500 000 IU intravenously in one hour) were significant alterations of coagulation parameters have been reported [27]. Therefore, hemorrhagic adverse events are less likely to be expected with this product. The low systemic exposure can also explain the fact that anti-SK antibody titers did not increase with treatment, contrary to the larger dose, intravenous administration [26].

Twenty hours of treatment were sufficient to achieve, in most of the patients, a complete and sustained improvement of all the main signs and symptoms of the illness. Ninety percent of the patients healed their hemorrhoidal crisis in approximately 5 days (pain relief before 48 hours). Other studies have reported longer healing periods with control standard treatments and other agents such as nifedipine or surgery [28-34]. The patients of this study faced inflammation, pain and an irreducible mass as the most important symptoms, also indicated by some authors [35]. The thrombolytic effect of the SK suppository on the local capillary structure could improve permeability and its action on the lymphatic local system could diminish the inflammation, exudates, and local edema and its action on blood viscosity, determines a rapid improvement in the first 24 hours after the product is applied [31]. In this study, although the sample was very small, typical complications of the disease such as anal abscess, vulvoperineal cellulitis, perianal fistula, among others, were not reported. Further, controlled trials are needed to confirm this initial efficacy data. Other doses and schedules should be explored as well.

The treatment of the hemorrhoidal crisis depends on the intensity of the signs and symptoms and can change in patients with thrombosis, important prolapse or profuse hemorrhage. In such cases, the surgical solution should be evaluated (hemorrhoidectomy, thrombectomy, ligature, sclerotherapy, infrared photoclotting, cryotherapy, therapy with laser). However, few randomized controlled trials have been performed to evaluate these procedures [6, 9-11].

In this work, only one patient did not improve his condition and needed thrombectomy. This patient presented hemorrhoidal thrombosis as first diagnosis, which improved without the need for analgesics. From the point of view of the clinical trial evaluation, he was considered a therapeutic failure due to the thrombus persistence. The surgical treatment of the hemorrhoids is indicated in grades III-IV, symptomatic, that have not responded to the conservative treatment to an associate illness (fissure, fistula, big skin flaps) and in the hemorrhoidal thrombosis [9, 35].

This is the first report in human of the recombinant streptokinase use in a rectal formulation (suppository).

17. Betancourt BY, Marrero MA, Jiménez G, Valenzuela C, García E, Hernandez-Bernal F, *et al*. Pharmacovigilance program to monitor adverse reactions of recombinant streptokinase in acute myocardial infarction. BMC Clin Pharmacol 2005;5(1):5.

18. Cáceres FM, Pérez H, Morlans K, Facundo H, Santos J, Valiente J, *et al*. Thrombolysis as first choice therapy in prosthetic heart valve thrombosis. A study of 68 patients. J Thromb Thrombolysis 2006;21(2):185-90.

19. López HP, Cáceres FM, Hernández KM, Sánchez HF, González N, Marrero MA, *et al*. Thrombolytic therapy with recombinant streptokinase for prosthetic valve thrombosis. J Card Surg 2002;17:387-93.

20. López-Saura P. Thrombolysis with recombinant streptokinase in Cuba. British Med J 2003;326(7386):448.

21. Francis CW, Marder VJ. Fibrinolytic therapy for venous thrombosis. Prog Cardiovasc Dis 1999;34(3):193-204.

22. Haas SK. Treatment of deep venous thrombosis and pulmonary embolism: Current recommendations. Med Clin North Am 1998;82(3):495-510.

23. Ansari A. Rapid lysis of deep vein thrombosis by low molecular weight heparin. Tex Heart Inst J 2000;27(1):74-5.

24. Estrada M, Hernández L, Pérez A, Rodríguez P, Serrano R, Rubiera R, *et al*. High level expression of streptokinase in *Escherichia coli*. Biotechnology 1992;10: 1138-42.

25. Naranjo CA, Shear NH, Busto U. Adverse drug reactions, in: Kalant H, Roschlau WHE (eds) Principles of medical pharmacology, 6ta ed., Oxford University Press, New York, 1998; p. 791-800.

26. Mainet D, del Rosario M, Toruncha A, Prats P, Valenzuela C, López-Saura P. Similar, more than 6-months persisted, antibody and neutralizing activity responses in patients with acute myocardial infarction treated with recombinant or natural streptokinase. Fibrinolysis Proteol 1998;12:301-9.

27. The TERIMA Group Investigators. Multicenter, randomised, comparative study of recombinant vs. natural streptokinases in acute myocardial infarct. Thromb Haemost 1999;82(6):1605-9.

The results indicate that it is unlikely that any systemic action of the thrombolytic agent (SKr), and thus adverse events on other organs or systems can appear. This will have to be confirmed in further studies.

Acknowledgements

Authors FHB, CV, SB and PLS are employees of the Center for Biological Research, which is part of the Center for Genetic Engineering and Biotechnology, Havana Network, where rSK is produced and the new formulation was developed. HB, AA, YB, RP and EM belong to the latter organization. The other authors have no conflict of interests. The study was financed by Heber Biotec, Havana (products, reagents) and the Ministry of Public Health of Cuba (hospital facilities and general medical care of the patients). The authors wish to thank Dr. Iraldo Bello Rivero, Olga Pantaleón Bernal, technicians Dunia Gómez Chávez, Maria de la C Hernández, Teresa Álvarez Padrón, Maria Isabel Nuñez and BSc, Magalys Cabro Cruz for their participation in the laboratory work. They also give thanks to the Dr. Luis Villasana Roldós for his advice.

28. Perrotti P, Antropoli C, Molino D, De Stefano G, Antropoli M. Conservative treatment of acute thrombosed external hemorrhoids with topical nifedipine. Dis Colon Rectum 2001;44:405-9.

29. De Paredes B, Demarez JP, Cauquil J. Analyse comparative des activités thérapeutiques de Cyclo 3 Fort et diosmine 450 mg associé à l'hesperidine 50 mg dans l'insuffisance veineuse des membres inférieurs. Étude en double insu auprès de 60 patients. Vie Médic 1990;6:226-32.

30. Lozes A, Boccalon H. Double-blind study of ruscus extract: venous plethysmographic results. Int Angiol 1984;3:99-102.

31. Cospite M, Cospite V. Treatment of hemorrhoids with Daflon 500 mg. Phlebology 1992;7 Suppl 2:53-6.

32. Gupta PJ. Suppositories in anal disorders: a review. Eur Rev Med Pharmacol Sci 2007; 11:165-70.

33. Granados J, Chávez C, López A, Rodríguez M, Yamamoto K. Eficacia de Fabroven en el tratamiento de la enfermedad hemorroidal aguda. Rev Mex Angiol 2005;33(2):62-6.

34. Giordano M. Randomized clinical trial on the use of a new molecule, formulated in suppositories, in the treatment of acute hemorrhoidal disease. Gazzetta Medica Italiana Archivio per le Scienze Mediche 2007; 166(4):121-6.

35. Balasubramanian S, Kaiser AM. Management options for symptomatic hemorrhoids. Curr Gastroenterol Rep 2003; 5(5):431- 7.

Received in April, 2010. Accepted for publication in September, 2010.