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Evaluation of a Streptokinase Suppository Formulation in a Rabbit Hemorrhoid Model

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Abstract

Recombinant streptokinase (rSK) is a protein with thrombolytic and anti-inflammatory effects widely use in some diseases like heard attack and hemorrhoid. The use of rSK rectal suppository application in animal model of hemorrhoid disease has not been report previously. The effect of this protein formulation in suppositories was evaluated in an experimental rabbit model based on the application of croton oil into the rectum. The animals were randomly distributed in four groups (four animals per group) of treatment: rSK, rSK+0.5% sodium salicylate, placebo and control, administered one suppository every six hours for 24 hours. At 24 hours, less inflammation was observed in the rectal mucosa of the animals treated with rSK, the lesion was reversed in 80% with the formulation containing sodium salicylate and 74% without sodium salicylate. In the placebo and control group, the reversal of inflammation was 53% and 30%, respectively. This lower inflammation in the groups treated with rSK was confirmed in histopathological studies. In the evaluation of haemostasis, the groups treated with rSK showed a reduction in fibrinogen levels and prolongation of thrombin time (higher in the formulation containing sodium salicylate). This evidence supports the rSK as suppository rectal formulation is active and pharmacologically acceptable to treat local inflammation and thrombosis in rectal area.

Keywords: Suppository; Recombinant streptokinase; Hemorrhoids; Croton oil

Introduction

The term, hemorrhoid is related to vascular cushions in the rectum epithelia which commonly produce clinical symptoms in healthy individuals. There are different risk factors like obesity, diarrhea, hereditary, constipation and others when the hemorrhoids become enlarged, inflamed, thrombosis, or prolapsed, they require emergent or surgical treatment. The pharmaceutical treatment is usually focused on decreasing inflammation and alleviating pain, but it does not dissolute thrombus, with subsequent recurrence of the pathological condition [1]. These preparations usually contain anesthetic and corticosteroid compounds in an isolated or combined form; few have been evaluated in clinical trials. These treatments, although initially they can alleviate the clinical manifestations, they only have to be applied during a few days, since they can cause loss of sensitivity of the skin, irritation, allergies, among others.

The specialized literature shows research aimed at treating hemorrhoids, which range from non-pharmacological therapy, natural and traditional medicine, to surgery. Very few controlled clinical trials are reported using conventional medical procedures. On the other hand, no studies have been found that evaluate the role of local anti-inflammatories or oral analgesics. In the case of creams, the majority of therapeutic trials that evaluate it are aimed at controlling pain after the hemorrhoid surgery. Despite this, its use in the general population is massive, due to the effect of self-medication, or because of its high medical prescription. There is not yet proven scientific evidence regarding its real clinical usefulness.

SK is an extracellular metallo-enzyme of 47 kd produced by beta-haemolytic streptococcus. It activates plasminogen through cleavage to produce plasmin. rSK as natural SK, converts plasminogen to the enzyme plasmin, which aids dissolution of blood clots [2]. Its effectiveness has been demonstrated in preclinical and clinical studies consequently, a new application has been looking with this molecule [3]. Thus, a formulation using suppository form in a hemorrhoid biomodel induced with crotton oil to evaluate the antihemorrhoidal activity in rabbits of this molecule is the aim of this study [4].

Considering the data above the thrombolytic effectiveness of the rSK, becomes in an attractive trends to search for new clinical applications of this molecule. A good option could be a solid formulation as suppository, for the treatment of hemorrhoid crisis (fluxion and/or thrombosis) since the same could generate benefits from a preventive approach, and it would decrease the need for invasive procedures, such as the surgical interventions.

Here we provide the first experimental animal model evidence on the favorable impact of the rectal administration of rSK, as a candidate to improve the hemorrhoids crisis.

Materials and Methods

Materials

Ethics: All animals used in this research were cared following the American Association for Accreditation of Laboratory Animal Care (AAALAC) guidelines, and the procedures were performed under approval CIGB's Institutional Animal Care and Use Committee (IACUC), according with the resolution no. 11/2013, Regulation no. 64/2013. Guidelines for the constitution and functioning of the Institutional Committees for the Care and Use of Laboratory Animals (CICUAL) of official regulatory Center for State Control of Medicines Equipment and Devices Physicians (CECMED), Cuba.

rSK formulation: The rSK was purchased by Biopreparation National Center (Habana, Cuba). Suppositories were obtained by the fusion method the mixture was dropped into 2 g cold suppository molds to be solidified and stored from 2-8°C to a final concentration

of 100000 UI/g at center for genetic engineering and biotechnology (Habana, Cuba) [5].

All the other chemicals used in the experiments were of analytical grade and were supply by Merck, Darmstadt, Germany.

The suppositories of the present study were prepared by mixing an oil base, with the necessary additives such as preservatives, protein stabilizers, and absorption promoters, maintaining constant stirring, until the mixture is gradually cooled to a temperature between 30 and 40 °C, which allows the incorporation of the thrombolytic agent (rSK) or the corresponding buffer. In all cases, took into account their use in a range of non-irritating concentrations of the rectal mucosa [6-8]. Cream color, torpedo-like shape suppositories with an average weight of 2.00 ± 0.02 g were obtained. The analysis of the formulated suppositories indicated positive for the presence rSK using chromogenic substrate except in placebo formulation [3].

Hemorrhoid induction Design and in vivo experiment in rabbits: Twenty-two female rabbits F1 (NZ x SGB), were purchased from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba). Animals weighing from 2-2.5 Kg with an average body weight of 2 Kg, not exceeding ± 20% were individually allocated at the animals' facility of the Center for Genetic Engineering and Biotechnology, Havana, Cuba, and maintained under conventional environmental conditions. Fasted for 12 hours prior to the experiment, but allowed free access to water. In all cases, the females were nulliparous and all animals were healthy and without clinical signs of pathological disorders.

Experimental design G1 (rSK), G2 (SK+SNa), G3 (Placebo), G4 (Control+): Rabbits were randomly (Aleatory system) divided in four groups: three of six animals that are group I: rSK suppository, group II: Suppositories of rSK+ sodium salicylate at 0.5%, group III: placebo suppository and the group IV Positive Control (Biomodel) with four rabbits. Before applying the suppositories, a control group (biomodel) of 4 animals was always selected.

To obtain hemorrhoid thrombosis, a model of hemorrhoids induced by croton oil in rabbits was taken as a reference [4]. Hemorrhoid were induced with an irritant solution, which contains one (1) part of water, four (4) of pyridine, five (5) of ether diethyl and ten (10) of croton oil at six percent (6%), at a rate of eight hundred microliter (800 µL) per animal. The optimal conditions were taken into account and the possibility of doing it in rabbits was studied because they are the species with anatomo-physiological characteristics of the anorectal portion more similar to human [9]. Once the animals were administered with ketamine at 40 mg/Kg of weight (s .c), a cotton swab soaked with inducer solution was applied to the anus rectal region for 60 sec. thus causing the lesion over time, its severity was detected by macroscopic observations and confirmed by histology. Before the administration of suppositories, rectal biopsies were obtained from four controls rabbit. In these animals, the presence of thrombus was evaluated by histological exams. Once the hemorrhoidal thrombosis biomodel was obtained, the suppositories were administered with a frequency of one every 6 hours for 24 hours, resulting in the repeated application of four suppositories per animal.

Blood collection and sample analysis: Blood samples were collected from the right femoral artery at baseline at 0, 3, 6, 12, and 24 hours after the suppository administration; and centrifuged at 3000 rpm for 10 min by a Hettich centrifuge, Germany. Serum was used to measure homeostatic and coagulation parameters; thrombin time from 5 to 10 seconds, and fibrinogen from 150 to 330 mg/dL [2]. Quantifying rSK was determined by ELISA after the last administration at 30, 45, 60,180 and 240 minutes [10].

Determination of fibrinogen and determination of thrombin time: Fibrinogen was measure by citrate blue blood coll tube, 2.7 mL, BD Cat. no. 363083 and analyzed in real time via an ACL 7000 (Beckman Coulter Diagnostics, Chaska, MN). The Reference values are from 150-330 mg/dL. Serum was separated and analyzed in real time via beckman coulter model #AU400 chemistry analyzer (Beckman Coulter, Franklin Lakes, NJ). Values of Reference were between five and ten seconds (5-10 s).

ELISA for the quantification of rSK: Polystyrene plates 96-well (MaxiSorp, NUNC) were coated with the monoclonal antibody SK1, at a concentration of 5 µg/mL in Carbonate-Bicarbonate buffer pH 9.6 (Na_2CO_3 0.015 M; $NaHCO_3$ 0.028 M) (100 µl per well) and incubated at 4°C for 16-18 hours. It was then washed 4 times with PBS-0.05% Tween 20 and blocked with 2% skim milk in PBS-0.05% Tween 20 for 1 hour at 37°C (200 µL per well). It was washed 3 times with PBS-Tween 20 at 0.05%, and 100 µl of the rSK standard curve (80, 40, 20, 10 and 5 ng/mL) in 1% milk buffer in PBS-Tween 20 at 0.05% was added. 100 µL of the serum dilutions, prepared in the same buffer as the standard, were added to the remaining wells. The curve, the positive controls and the samples were applied with three replicas. The plate was incubated 1 hour at 37°C and then washed six times. The anti-rSK conjugate was added in the working dilution, diluted in 1% milk buffer in PBS-0.05% Tween 20. It was incubated 1 hour at 37°C and the plates were washed 6 times. The substrate, 0.05% orthophenylenediamide and 0.05% hydrogen peroxide diluted in phosphate-citrate buffer pH 5.5 (0.048 M citric acid; 0.1 M Na_2HPO_4) were applied. The reaction was stopped with 50 µL of 2M sulfuric acid. Absorbance at 492 nm was determined in an ELISA plate reader.

Clinical evaluation: The clinical observations were carried out from the moment 0, when the insertion of the suppositories by anorectal route was executed, during a period of twenty-four hours (24h). There was used a modification of the draize system, which bases on carrying out a count of the clinical symptoms and signs that allow evaluating the dermal irritation and the mucous in general. To that end, there were selected the three factor with the highest incidence in the clinical observations. Any adverse sign attributable or not to the product were evaluated (Table 1).

Inflammation	1-Reddening, 2-Slight to moderate, 3-Severe with secretion
Formation of gallbladders	1-In formation, 2-Scarce, 3-Abundant, well defined, with or without secretion
Perianal hardening	1-Slight, 2-Moderate, 3-Severe
Others	1-Ulcer, 2-Necrosis

Table 1: Count system for the clinical evaluation of the effectiveness of the response of the suppositories, given three fundamental factors.

Morphology test of rectal tissues: All animals were euthanized by concussion after administration of ketalar (40 mg/Kg) for sedation and recto anal tissue (20 mm in length) was isolates and weighted. Macroscopic and microscopic observations, as well as, histological examinations were carried out after 24 hours in this study.

Statistical analysis: For all the variables, there were verified per time and group of treatment, the suppositions of approximation by a normal distribution (Shapiro-Wilk tests) and of homogeneity of variances (levene test). In the cases when such suppositions were satisfied, there was used a parametric Analysis of Variance (ANOVA). In the opposite cases, there was used the non-parametric alternative (Kruskall-Wallis test), taking into account the Bonferrony correction for the type-I error (alterations of the anorectal mucous: alpha=0.008; time of thrombin: alpha=0.025). In the cases in which there were detected statistically significant differences between the groups, there was applied the Tukey´s test of multiple comparisons. Also, there were made paired comparison in each group with respect to the beginning, using the paired t test or the Wilcoxon test, depending on the fulfillment of the supposition of approximation by a normal distribution, considering also in this case, the correction of the bonferrony error (alpha=0.0125).

Results

For the selection of the dose to be used in the suppositories, the extensive information collected regarding heberkinasa was taken into account by different inoculation routes for the pathologies registered as thrombolytic (X), and where if different dose levels were analyzed, it was believed convenient to take as reference the dose administered in humans for the treatment of deep vein thrombosis (3148.85 IU/Kg of weight) since hemorrhoidal thrombosis is classified as venous thrombosis of similar etiology and taking into account that rSK is a species-specific protein that unlike humans, where the rSK-Plasminogen complex is a 1:1 ratio in rabbits, it is 10:1.11, rSK suppositories were used with at 100000 IU/g.

To obtain hemorrhoid thrombosis, a model of hemorrhoids induced by croton oil in rats was taken as a reference the optimal conditions were taken into account and the possibility of doing it in rabbits was studied because they are the species with anatomo physiological characteristics of the anorectal portion [4]. More similar to human once the animals were administered with ketamine at 40 mg/Kg of weight (s .c), a cotton swab soaked with inducer solution was applied to the anus rectal region for 60 sec. thus causing the lesion over time, and its severity was detected by macroscopic observations and confirmed by histology.

Table 2 shows the degree of alteration of the anorectal mucosa, according to the average clinical observations of each study group over time.

Observations Time	Group				P (ANOVA)
	I	II	III	IV	
0h	92.5±7.6	94.3±4.3	90.3±6.4	95.2±9.5	0.67
1h	80.0±12.6	73.3±20.6	70.7±6.5	85.0±12.9	0.402
3h	56.0±14.4	46.7±16.3	58.3±7.5	78.8±8.5	0.019*
6h	48.0±13.6	28.3±16.0	53.7±9.1	75.5±6.4	0
12h	30.0±6.3	23.3±12.1	36.7±6.0	67.5±5.0	0
24h	18.3±7.5	14.3±4.8	36.7±8.2	65.2±4.1	0

Table 2: Clinical observations of the results obtained in the counting system (media± DS).

Clinical observations made during the experimental phase after administration showed the presence of edema, destruction of the mucosal epithelial cells, necrosis, vesicles, discharge, some prolapses and evident inflammation. The statistical processing shows that there are significant differences between the placebo and the positive control with respect to the formulation of suppositories of rSK and rSK + SNa at 0.5% and that there is no statistical significance between them at 24 hours.

However, from the biological point of view, is important to highlight that in the case of the animals that were administered with the suppositories of rSK (group I), it was not until ten to twelve hours (10-12h) had elapsed, that there began, in a palpable manner, the reversion of the lesion and even the improvement of the animal in terms of its disposition to consuming food and water, and of the increase of the mobility inside the cage, with respect to the animals of the group of treatment with suppositories of rSK + sodium salicylate at 0.5%, in whose case, only three hours (3h) after the administration, they exhibited the recovery of the parameters above mentioned, achieving the complete recuperation. This aspect was more evident from the six hours (6h), in coincidence with the statistical result. The above suggests the effect on the lesion and, therefore, in the general health condition that the animal exhibits after the administration of the suppository of rSK + SNa at 0.5%, which results in an important level of animal well-being. Based on what has been previously stated, we consider that the non-significance between groups I and II, at the twenty four hours (24h), does not constitute a relevant piece of information, which is also maintained in the results shown below with respect to the pharmacological parameters evaluated, specifically the values obtained in the time of thrombin at the twenty four hours (24h),which evidence significant differences between groups I and II (Figure 1).

The Figure 1 show the alteration level of the anorectal mucous, based on the average clinical observations of each group of study over time.

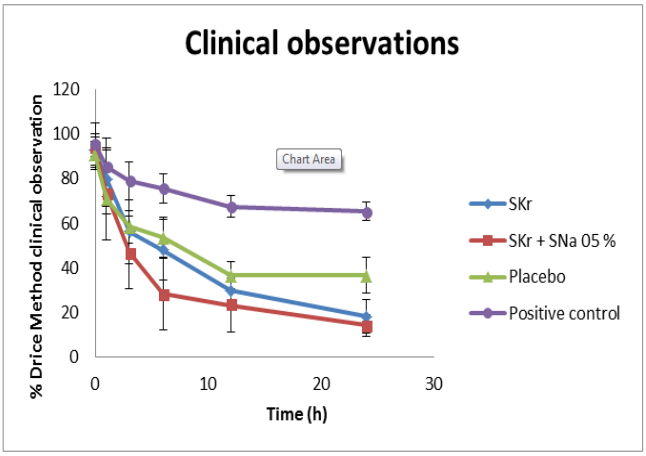

Figure 1: Reversion of the lesion per clinical observations.

It is important to point out that the effect of the SNa at 0.5% turns out to be relevant in the formulation of the suppository of rSK+SNa at 0.5%, given the characteristics of these substances of facilitating the absorption at mucosal level, especially the anorectal mucous, which has been nominated by several authors as the route of preference for the administration of peptides and proteins [8].

This function favors a decrease in the fluidity of the membrane, expansion of the dimension of the intercellular space with solubilization of the mucosal membrane, increment in the fluidity of water and reduction of the viscosity of the mucous layer, which facilitates its adhesion to the entire mucosal surface. The changes that take place in group III, from the beginning, may be related, to the presence of the sodium salicylate promoter in the formulation, which, aside from facilitate a rapid absorption of the substance of trial via anorectal route, has other associated effects like the anti-inflammatory; this sign being predominant in the clinical findings, in all the groups of animals.

Determinations of fibrinogen

The fibrinogen is the plasmatic protein of the blood responsible for the coagulation. Under the catalytic action of the thrombin, the fibrinogen is transformed into the insoluble protein, fibrin, which is the structural element of the blood coagula or thrombus [11]. This evidences the presence of coagulation. These values can also be higher in the presence of acute inflammation [12].

The results of the study showed that there are significant differences among all the formulations studied. There was a higher significance in the animals to whom there was administered the formulation of suppository of rSK+SNa at 0.5% (group 2) and of suppository of rSK (group 1), with respect to the positive control group. However, taking into consideration the clinical findings previously referred to there is evidence of a marked reversion of the lesions in the anorectal tract in the animals treated with rSK+SNa at 0.5% (group 2). Based on the results obtained in the determination of the values of Fibrinogen at the twenty-four hours (24h), there is observed a decrease with respect to the initial day of the first treatment with the suppository of rSK+SNa at 0.5%, which indicates and confirms the effectiveness of the product evaluated.

The units of average difference between the cero and the twenty four hours (0-24h), in the different groups, are clinically relevant for groups 1 and 2. They represent from a sixty three point three to a sixty eight point twelve percent (63.3-68.12%) of the fibrinolysis in the zone of the lesion, while for the placebo and the positive control groups, they only represent between a three and seven percent (3-7%), which it was consider to be closely related to the absence of the active pharmaceutical ingredient (SKr) in the formulations of suppositories administered to the animals of groups III and IV. It is important to point out that the variation of each group between the beginning and the end of the treatment (0-24h) constituted decisive points of sampling for the determinations of fibrinogen (Table 3).

Treatments	0h (mg/dL)	3h (mg/dL)	6h (mg/dL)	12h (mg/dL)	24h (mg/dL)	Difference 0-24h (mg/dL±DS)
Control without treatment	252	-	-	-	-	-
SKr	618.17±3.061	503	487	468	421	195.5±6.37
SKr + SNa al 0.5%	625.33±3.55	498	464	409	396	229.33±8.52
Placebo	600.50±7.58	595	587	570	560	40.83±9.15
Positive control (Biomodel)	611.50±6.95	612	604	599	592	19.25±8.99

Table 3: Fibrinogen values in each group.

Determinations of thrombin time

The Time of Thrombin is a trial that evaluates the formation of fibrin. It is determined by adding into the plasma a fix quantity of thrombin and determining, afterwards, the time of coagulation. This method omits all the steps of the cascade of coagulation in the presence of the thrombin. In this way, there is ruled out any anomaly of the same, given the formation of fibrin in an artificial manner.

The prolonged value of the same can respond to the presence of products of degradation of the fibrin, which allow us the use of this parameter as an indicator of fibrinolysis. Taking into account these aspects and the statistical results obtained, at the twenty-four hours (24h) there are detected statistically significant differences among the groups, specifically among the even groups, except between groups III and IV. This is an expected behavior, taking into account that the time of thrombin only prolong in the presence of products of degradation of the fibrin. However, in groups I (suppositories of SKr) and II

(suppositories of rSK+sodium salicylate at 0.5%), there are significant differences evidenced, which indicates that in the anorectal zone, location selected for the model of hemorrhoids, the thrombolysis took place, which confirms that the formulation with promoter of SNa at 0.5% is an option suitable for the treatment of hemorrhoid thrombosis in rabbits.

Thrombin time evaluates fibrin formation so prolonged values have been seen due to the presence of degradation products, which allowed to use this parameter as a fibrinolisis indicator. Statistical results showed the effectiveness of rSK suppositories indicating degradation fibrin in the ano-rectal region with hemorrhoid disease because of the active pharmaceutical ingredient.

After rectal administration, passive transport is the main absorption mechanism, and depends mainly on the molecular mass, the fat solubility and the degree of ionization of the molecules [13].

Depending on the chemical structure, drugs can pass through the rectal mucosa by absorption through the epithelial cell or through the interconnecting junctions of the mucosal cells. Due to the poor penetrability of peptides and proteins through the mucosal route, it was decided to evaluate SNa at 0.5% as absorption promoters [14].

The promoting effect of SNa, is due to the increase in absorption through the paracellular pathway, acting directly on the calcium (Ca2+) found on the surface of the membranes or decreasing Ca2+ cytosolic, which allows the opening of pores in the membrane [8].

These results suggest that the presence of SNa favored the recovery of the injury and with it, the body condition of the animal, which is why this finding was relevant for the formulation. SNa has been reported to promote an increase in membrane fluidity, expansion of the dimension of the intercellular space with solubilization of the mucosal membrane, increase in water flux, and reduction in the viscosity of the mucous layer. All this facilitates the absorption of the rSK in the mucosal surface [15].

Another advantage that the use of SNa could have in the formulation is related to its ability to induce urokinase-type plasminogen activator [16]. Some benefits have been reported in the derivatives of salicylates because their effect on the mucosa is reversible and occurs without substantial damage to the epithelial membranes [15].

It is important to highlight that for a local successful thrombolysis, it is essential certain level of systematic activation of the fibrinolytic system, for the dissolution of the thrombus; but the same does not entail any complication, as stated in the literature (Figure 2).

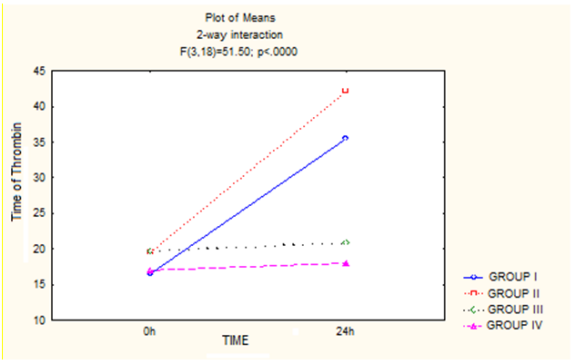

Figure 2: Results of the time of thrombin.

Analysis of the walls of each group (corrected alpha=0.0125).

As we previously stated, the formation of fibrin is activated in an artificial manner. This allows us to assert that the results obtained are the effect of a local action, corroborated by the previous experiments in which there was evidenced the non-penetrability of the active principle into a systemic route.

Quantification of the rSK in serum of rabbits treated with suppositories

In the serum of the rabbits, there was not reported recombinant rSK at the levels detected by the ELISA technique. This implicates that there is no peripheral circulation, that is, the level of absorption is much lower than 5 ng/ml or it does not exist.The data is expressed in ng/mL. No rSK was observed at 30, 45, 60, 180 and 240 minutes by chromogenic substrate using rSK suppository administration indicating no rSK in blood. This result has been obtained in other studies [17].

Histology results

The histological results obtained in the model of hemorrhoid thrombosis in rabbit which was described in the study as positive control (Group IV) is described below. (Figures 3 and 4).

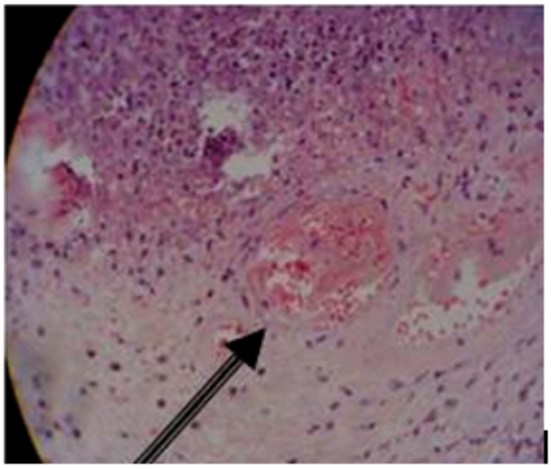

Figure 3: Thrombus partially occlusive at the 30th hour.

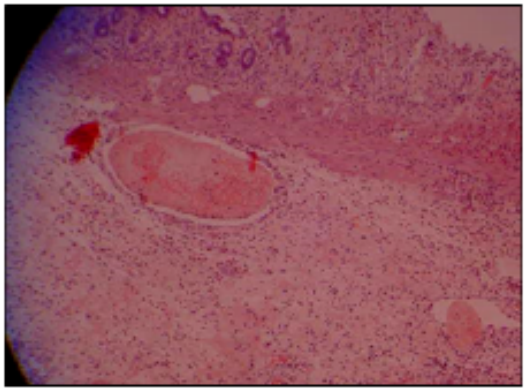

Figure 4: Presence of thrombus in the submucosa at the 48th hour.

By histological evaluations the samples of the groups of animals treated with the different suppositories formulations, there was demonstrated the presence of edema of the submucosa, damage of the anorectal mucous, infiltration of fibrin, high level of necrosis, hemorrhage and scarce microthrombuses.

On the other hand, in the group administered with placebo, as expected, these signs were not observed and the inflammatory response was much lower, with the predomination of a tissue granulation which corresponds to a lower intensity of the damage of the anorectal mucous.

The non-treated Group IV comprised of the positive control (bio model), aside from allowing having an important clinical parameter of comparison, possibilities knowing whether there existed any crossed reaction with the physiological mechanism of elimination of the thrombus (Figure 5).

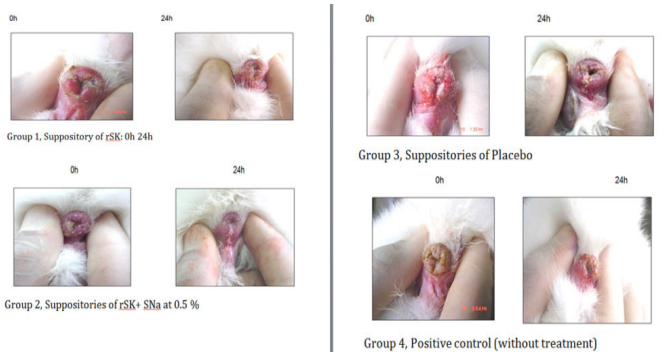

Figure 5: Macroscopic observations.

Discussion

Protein drugs are increasingly becoming a very important class of therapeutic agents as a result of our gaining more understanding of their role in physiology and pathology as well as the rapid advances in the field of biotechnology/genetic engineering [18].

The route of administration has a significant impact on the therapeutic outcome of a drug. These drugs are generally not suitable for oral administration, since they are poorly absorbed and easily degraded by proteolytic enzymes in the gastrointestinal tract [19].

The rectal delivery of protein drugs provides the advantage of greater systemic bioavailability. Additional advantage is the avoidance of first-pass elimination [20].

Hence, it is very convenient in all types of patients, especially those with mental illnesses and in child therapy, due to its rapid and direct absorption due to the high irrigation of the administration area.

For the aforementioned reasons and especially for its use as a thrombolytic agent, at the center for genetic engineering and biotechnology, a solid formulation in the form of a suppository of Skr was developed for its use in the treatment of hemorrhoidal thrombosis, a disease that affects a high percentage of the world's population, as described above.

There are multiple formulations on the market in the form of suppositories or rectal ointments for the treatment of hemorrhoids, which contain anesthetics, anti-inflammatories and antipruritics such as Ercal, Sheriproct, Ultraproct, Xyloproct Proctaxid and Proctoglyvenol, drugs that are mainly aimed at reducing inflammation and the pain of the pathology.

The anti-inflammatory effect is achieved when the plasminogen transformed into plasmin digests the fibrinogen, fibrin or both present in the inflamed areas or in the clots, which smooth thus favoring their drainage and therefore reduces edema and inflammation [21].

Considering the thrombolytic effectiveness demonstrated in preclinical and clinical trials of recombinant streptokinase (SKr) produced in CIGB, a solid formulation in the form of a suppository for the treatment of hemorrhoidal thrombosis, it could generate benefits from a preventive approach and reduce the need for procedures invasive.

Experimental biomodels are necessary for the study of these formulations and their mechanisms. The rabbit hemorrhoid model obtained allowed the formulation to be evaluated in rabbits. This was obtained by non-invasive methods.

There are similar non-invasive experimental models of venous thrombosis in rats and mice, but none is induced in rabbits, with the advantages that this species suggests. Considered an excellent model for testing skin and eye irritants, due to its high sensitivity for these products [10].

More recently, the stasis model in mice was published, which induces consistent thrombus size and a quantifiable amount of thrombus, the latter limiting the number of mice required for the quantification of venous thrombosis consistent with the principle of substitution, reduction and refinement of animals in research [22].

Another model published in rabbits is the intimectomy venotomy model that is valid and produces thrombosis in 100% of cases, however, surgical revision, excision of the anastomosis and resuturing of the vessels are required, with the complications and expenses that these procedures presuppose.

Blood coagulation involves the formation of fibrin by the interaction of more than 12 proteins in a cascade series of proteolytic reactions [23]. “Non-fibrinespecific” activators such as streptokinase (SK converts both circulating and bound plasminogen to plasmin clot, leading not only to fibrin lysis in the clot, but also to significant systemic fibrinogenolysis, fibrinogenemia, and elevation of circulating fibrin degradation products [24].

Under the catalytic action of the thrombin, the fibrinogen is transformed into the insoluble protein, fibrin, which is the structural element of the blood coagula or thrombus. This evidences the presence of coagulation. These values can also be higher in the presence of acute inflammation [25-28].

Mechanisms corroborated in our study, in which it was observed sixty three point three to a sixty eight point twelve percent (63.3-68.12%) of the fibrinolysis in the zone of the lesion, while for the placebo and the positive control groups, they only represent between a three and seven percent (3-7%), which it was consider to be closely related to the absence of the active pharmaceutical ingredient (SKr) in

this formulation. It was also observed the prolonged value of the same can respond to the presence of products of degradation of the fibrin, which allow us the use of this parameter as an indicator of fibrinolysis. As we previously stated, the formation of fibrin is activated in an artificial manner. This allows us to assert that the results obtained are the effect of a local action, corroborated by the previous experiments in which there was evidenced the non-penetrability of the active principle into a systemic route. In correspondence with the values obtained in the quantification of the rSK in Serum of Rabbits Treated with Suppositories, where we confirmed there was not reported recombinant rSK at the levels detected by the ELISA technique the in the serum of the rabbits. Very favorable result considering the main complication of rSK treatment, which is hemorrhage, which is related to the dose and the route of application [29,30].

By histological evaluationing histologically the samples of the groups of animals treated with the different suppositories formulations, there was demonstrated the presence of edema of the submucosa, damage of the anorectal mucous, infiltration of fibrin, high level of necrosis, hemorrhage and scarce microthrombuses which corresponds to a lower intensity of the damage of the anorectal mucous compared to the non-treated group, comprised of the positive control (bio model), aside from allowing having an important clinical parameter of comparison, possibilities knowing whether there existed any crossed reaction with the physiological mechanism of elimination of the thrombus [31]. The resulting conclusion was that the effect and the findings obtained were inherent to each formulation.

Above results suggest that rectal suppository is worth developing as an alternative dosage form of rSK to the conventional parenteral preparations which need sophisticated or invasive treatments.

Conclusion

The study demonstrated the first experimental study that the solid formulation in the form of suppository of recombinant rSK with promoter of sodium salicylate at 0.5%, turned out to be effective in the experimental treatment of hemorrhoids in rabbits, taking into account the clinical aspects evaluated, since it allowed a more rapid reversion of the lesion (3h) in the experimental model trialed.

The results of the statistical processing in the clinical observations of the suppository of SKr + SNa at 0.5%, show an adequate effectiveness taking into account the significance of said product with respect to the placebo and the positive control, which is corroborated with the hemostatic values obtained in the determination of fibrinogen and thrombin time in which statistical significance is evidenced between groups 1 and 2 at 24 hours. Indicative these are favorable for its indication proposal in humans.

Likewise, the local application of these suppositories can generate benefits, from a preventive approach, and in many cases, it can prevent the surgical procedure and, consequently, it can contribute to the decrease or prevention of the complication of the hemorrhoidal thrombosis.

Conflict of Interest

The work described here has not been published previously and none of the authors have any conflicts of interest to declare that limit the publication of this paper in this journal.

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