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Scientific & Clinical Evidence of Combining an Anti-Oedematous Solution with a Specific Pro-Inflammatory Cytokine Inhibitor is the Only Approach for the Treatment of Hemorrhoids

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Abstract

Background

Hemorrhoids are dilated, oedematous and inflamed veins of the venous plexus of the anal opening. Treating hemorrhoids therefore requires reducing the oedema as well as the concentration of pro-inflammatory cytokines present on the hemorrhoidal surface. In the absence of any treatment designed to act on these causes, currently there is no satisfactory treatment for hemorrhoids.

Objectives

The aim of our research was to identify and to neutralize inflammation-inducing cytokines and to find a non-irritant anti-oedematous solution to reduce the piles' volume.

Materials & Methods

Pro-inflammatory cytokines were identified *in vitro* and polymers were used to neutralize their activity. These specific cytokine-blocking polymers were then incorporated in VB-Gy, a glycerol-based, highly osmotic, non-irritant solution capable of attracting hypotonic liquid from the oedematous plexuses. This solution was then filled in sprays (liquid) or tubes (gel) for external or internal application on the hemorrhoidal surface. A clinical trial was conducted on 20 placebo and 31 active treatment patients to evaluate the effects over a 6-week treatment period. Placebo patients were treated identically (3-4 topical applications per day) with a saline solution to compare the results.

Results

The dual approach of inhibiting pro-inflammatory cytokines and reducing oedema simultaneously was 2-3 fold more effective in providing symptomatic relief and promoting hemorrhoidal regression compared to the placebo saline solution.

Conclusions

Simultaneously reducing oedema and blocking inflammation may constitute an ideal curative treatment for internal and external hemorrhoids in the future.

Keywords: Hemorrhoids; Oedema; Inflammation; MMPs; Cytokines; Topical; Inhibitors; Polymers; VB-Gy; Hypertonic; Clinical; Treatment; Efficacy

Introduction

Piles or hemorrhoids affect nearly 4.5% of the world's population and, whether painless or painful, are manifested as rectal bleeding associated with the presence of oedematous blood vessels at the level of the anal opening, and the presence of blood in the feces [1]. Although hemorrhoids are not a life-threatening disease, they cause considerable discomfort and worsen considerably the quality of life of the patients.

The exact etiology of the development of hemorrhoids is still unclear but it is now widely believed that this is related to the disintegration or deterioration of the supporting tissues of the anal cushions leading to the sliding of the anal canal lining, eventually causing the protrusion of dilated venous plexus [2]. The lesions may be located either below (internal) or above (external) the anal dentate line. Compression of vessels leads to poor venous return and the development of an oedematous hemorrhoidal plexus. Poor blood circulation in the venous plexus progressively leads to vascular thrombosis, degeneration of the collagen fibers and fibro-elastic tissues, and the formation of inflammatory oedematous hemorrhoidal masses [3].

The vascular damage triggers a local inflammatory cascade with the liberation of various matrix metalloproteinases (MMPs), cytokines and growth factors, as a response to the injury. Healing of an inflammatory tissue normally requires anti-inflammatory cytokines, but in case of hemorrhoids, the oedematous vessels do not allow rapid reabsorption of the swollen or prolapsed tissue, leading to an uncontrolled production of anti-inflammatory as well as pro-inflammatory cytokines [4]. All cytokines and extra cellular matrix-destroying MMPs are proteins in nature. Scientific evidence shows that in chronic injuries, such as non-healing hemorrhoids, the concentration of pro-inflammatory cytokines exceeds that of anti-inflammatory cytokines, causing the healing process to stagnate. Major anti-inflammatory cytokines include interleukin (IL)-1 receptor antagonist, IL-4, IL-10, IL-11, IL-13, and IL-18. Leukemia inhibitory factor, interferon-alpha, IL-6, and transforming growth factor (TGF)-β are categorized as either anti-inflammatory or pro-inflammatory, depending upon the status and chronicity of the lesion [5,6]. Pro-inflammatory cytokines are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions. It is postulated that the key pro-inflammatory cytokines are IL-1β, IL-6, and TNF-α [7]. Therefore, an ideal treatment should not only reduce the oedema but should also selectively block or remove the pro-inflammatory cytokines from the hemorrhoidal surface so as to offer instant symptomatic relief and progressive lesion healing.

Currently, there is neither any anti-edematous drug nor a treatment to block the pro-inflammatory cytokines specifically. This is the reason why only surgical or symptomatic treatments such as saline water are used to hydrate the piles surface along with the use of antiseptics to minimize bacterial contamination and some gels to protect the piles surface from drying and irritation [8]. Among the most effective treatments available, cleaning the hemorrhoidal surface with saline water solutions still remains one of the relatively effective, and safe, remedies. Saline solutions form a thin hypertonic film over the oedematous hemorrhoidal surface, attracting hypotonic liquid, and thereby cleaning and hydrating the hemorrhoid, and minimizing the concentration of pro-inflammatory cytokines, consequently reducing inflammation and oedema to some extent. Unfortunately, despite being a safe and inexpensive topical treatment, saline or salt solutions are not commonly used because of their poor efficacy due to instant dilution with the hypotonic liquid and strong irritation in case the concentration of NaCl is increased above 3%., whereas other chemicals that might have been used as hypertonic solutions are toxic and irritant to live cells at active concentrations [9] and cannot be used. Honey could have been an option too, as it is hypertonic and not irritant, but due to its saturation in sugar, honey is not highly osmotic, and upon drying may form crystals on the hemorrhoid surface, causing irritation and pain. Honey may also be contaminated with pesticides, heavy metals, bacteria, pollens and other allergens, and, furthermore, it is not filmogen, thus requiring frequent applications.

Therefore, the aim of our research was to find a highly osmotic but non-irritant and non-toxic solution for topical application to reduce oedema, and to incorporate inhibitors of specific pro-inflammatory cytokines in this solution so as to stop the inflammatory cascade, this combination representing the only scientific and logical approach for the treatment of hemorrhoids [10,11].

Materials and Methods

Preliminary Research

Selecting a Hypertonic, Non-Irritant, Osmotically Active Ingredient: The first step was to find a hypertonic liquid for topical application that is cell-friendly, non-irritant, resistant to liquid flow, and substantially more osmotically active than sea water or honey.

After testing different hypertonic substances for their potential cellular interactions and cellular toxicity using *in vitro* cell culture models, we identified some natural, cell-friendly, hypertonic ingredients: plant-based (plant resins), sugar-based (ex. sucrose, glucose, fructose, mannitol, saccharose), or sugar–alcohol-based (ex. glycerol or glycerine), solutions.

Finally, a glycerol-based solution (VB-Gy) was conceived as glycerol is natural, homogenous, contaminant-free, viscous, cell-friendly, non-irritant to sensitive mucosa, and 18 times more osmotically active than sea water. VB-Gy is a highly hygroscopic solution due to its high osmolality (3500 ± 200 mOsm/kg compared to 1000 ± 100 mOsm/kg for 3.2% NaCl). This solution was further rendered filmogen for better resistance to dilution by adding a few specific polymers capable of binding with the glycerol molecules [11].

Isolation of Plant Polymers as Protein Antagonists

Initially, 86 plants or parts of plants were selected based on their tannin (polymer) content to prepare extracts according to the method of Giner-Chevez et al. [12]. In short, the initially selected polymers were obtained from 86 tannin-rich plants/fruits with an aqueous organic solvent containing 70% acetone and 30% water. The extracts were then successively passed through Sephadex LH-20 columns by progressively increasing the volume of methanol (60x88.5 cm), and the intended fractions were eluted to produce a dry solid. The product was identified by mass spectrometry. The extracts used for experiments contained mainly 60-80% epicatechin, catechin B1, B2, B3 and C1 polymeric fractions and were used as plant polymers.

Research for the Cytokines present on the Hemorrhoidal Surface

The proteins (pro- or anti-inflammatory cytokines) present on the hemorrhoidal surface have been identified through the slightly modified method of Shrivastava et al. [13]. In short, vascular smooth muscle cell cultures representing to a large extent the vascular cells present on the hemorrhoidal surface were isolated from rabbit aorta and were grown in 5% CO_2 at 37°C in 5% foetal calf serum (Labtech, France) in 24-well tissue culture plates (Corning, USA) for the first 24h to obtain 30-40% cell monolayer. After 24h, cell culture medium was replaced by a new serum-free medium supplemented with different non-cytotoxic concentrations of purified individual cytokines (Anaspec Inc, US) to evaluate their effects on cell growth, indicative of the cellular toxicity of these cytokines on the hemorrhoidal surface. Cytokines which individually reduced cell growth by ≥ 20% were then associated, at half the initial concentrations, in various combinations so as to select the associations causing maximum cellular toxicity (80-100%). Cell growth was measured at 72h using MTT vital stain, and results were compared with control cell cultures to which no cytokine had been added. The most cytotoxic association of cytokines was identified and termed "VB-cytokines".

Evaluation of Cytokine-Neutralizing properties of Polymers

The anti-cytokine activity of different plant polymers was measured using the method as described by Shrivastava et al. [14]. Briefly, a fixed concentration of each polymer (50µg/ml) or an association of polymers (25µg/ml each) was pre-incubated for 1h with VB-cytokines at a fixed non-cytotoxic concentration of 0.5µg/ml. After 1h pre-incubation, the polymer–cytokine suspension was exposed to the SMC cell cultures and cell growth was measured. Enhanced cell survival compared to cells exposed only to VB-cytokines indicated cytokine neutralization by the polymer(s). Different polymers were tested to select the best polymeric association neutralizing 90-100% VB-cytokines present on the external or internal hemorrhoidal surfaces.

According to the desired consistency of the final product, these polymers were then incorporated in the filmogen VB-Gy solution. Product viscosity was then adjusted with water and the resulting preparations were filled in 30ml aluminum sprays (more liquid) or in 10 and 50ml plastic tubes (more viscous solution), and identified as Pileseptine spray and Pileseptine gel, respectively.

Those products were then clinically evaluated to study their effects on external and internal hemorrhoidal parameters.

Clinical Trial to Verify Safety and Efficacy of Resulting Preparations

Aim of the Study

The primary objective of this study was to evaluate the efficacy of Pileseptine spray on symptoms and regression of external hemorrhoids. The secondary objectives were to observe the effects of Pileseptine gel (concentrated solution) on internal hemorrhoids and anal lesions, in a few patients, to analyze eventual side effects, if any, and to evaluate investigator's and patient's comments on the product efficacy, mode of application, and mode of delivery.

Study Design

This study was a single-blind, placebo-controlled, multicentre, pilot prospective clinical trial which was performed at three different hospitals in India.

Setting and Participants

The study protocol has been approved by Institutional Review Board and Independent Ethical committee agreed by the Indian Council of Medical Research (ICMR) that is designated for observational clinical trials on OTC products in each hospital. These committees follow ICH guidelines for GCP and the principles laid down in the declaration of Helsinki and subsequent amendments. All patients gave their written informed consent.

Patient Enrollment

The patients were enrolled during the screening visit by the investigator based on clinical signs of hemorrhoids. All the patients included in the clinical study underwent careful physical examination and records were maintained with detailed clinical history. Each patient's history was recorded on a case history sheet and examined for any other previous disease history. At the start of the study, examination of blood, stools and urine was performed to exclude patients having gastro-intestinal, liver, renal or other diseases that may affect study outcome.

Key Inclusion and Exclusion Criteria

Inclusion criteria were: patients between 10 and 80 years old; presenting external or internal hemorrhoids or hemorrhoidal lesions; having no dermatological or other serious diseases like cancer; not under any medical

treatment in the last 3 weeks; not having taken antibiotics, anti-histaminic or steroid drugs in the last 7 days; ready to participate in the study by signing the written consent; ready to fill out the questionnaire and attend the initial and final medical visits at the hospital; and able to read and write.

Number of Patients

It was decided to conduct the study on at least 20 patients in each group.

Product Application

Products were supplied by the sponsor as sprays (30ml) or tubes (10 and 50ml tubes containing a gel). For external hemorrhoids, the product was sprayed directly over the surface of the hemorrhoids, 3-4 sprays per application, 2-4 times per day. For internal hemorrhoids, patients were asked to pour about 1-2 ml of gel on their finger and apply the gel directly over the internal hemorrhoid or hemorrhoidal lesion, 2-4 times per day. The treatment was pursued for a maximum period of 6 weeks or up to complete recovery.

Follow up

Follow up was conducted on the 1st and the last day by the CRO's (Clinical Research Organizer) at investigational site. During the intermediary period, patients were asked to come for a check-up with the CRO once a week or at least once every two weeks. When patients were not able to come for check-up (± 1 day), they were asked to fill out the observation table employing a 0-10 scoring system for each parameter.

Parameters Studied

Each patient was asked to evaluate and to record the intensity of hemorrhoidal symptoms/parameters on a 0 to 10 scale. 0 represented no disease/symptom, and 10 very severe symptoms. The scores were given at weekly intervals by the patient (and the medical professional on the 1st and the last week of treatment, only) for the following parameters: oedema; pain intensity; bleeding; redness around lesion; burning sensation; itching intensity (pruritus); feeling of constipation; feeling of weakness; hydration of piles; overall comfort; and the comments from the patients and investigators were also collected at the end of the study.

Statistical Analysis

Due to the small number of patients, data were analysed using the Wilcoxon Sign Rank test when the normality assumption was false. Descriptive statistics i.e. mean standard deviation (SD), minimum and maximum frequency distribution were used for the analysis of the demographic details, clinical evaluation, and medical history parameters.

Results

Identification of Pro-Inflammatory Cytokines on the Hemorrhoidal Surfaces

Among 18 selected cytokines tested individually, only 7 cytokines (MMPs 1, 2, 3, 7, 8, 9 and TNF-α) caused cellular damage *in vitro*. The cellular toxicity ranged between 12-22% indicating that although individual cytokines may exert cellular damage or inflammation, their toxicity on vascular cells is not too high. When these cytokines were associated with each other at half the concentration, we observed that the cytotoxicity increased progressively and produced a nearly additive effect. Maximum cytotoxicity (52%) was observed with the associations of MMPs 2+3+7+9 (*MMP-i*) and while a cellular toxicity score up to 59% was observed with MMPs 1+3+7+8+9+TNF-α association (*MMP-e*), the associations of MMPs 1+2+7+9 or 1+2+3+7+8+9+TNF-α did not induce higher cytotoxicity compared to the previous associations.

Selection of Polymers capable of Neutralizing *MMP-e* and *MMP-i*

When 34 natural polymeric substances obtained from plants were pre-incubated at different non-cytotoxic concentrations with MMP-e and MMP-i, we observed that only 7 polymeric substances were capable of inhibiting MMP-e and MMP-i -induced vascular SMC cytotoxicity between 65-80%. Among these 7 polymeric extracts, 2 extracts (codes V. ma and V. v) were particularly active, inhibiting both MMP associations up to 65-74%. Finally we found that a specific combination of these two polymers at a concentration of 0.623% was able to block MMP-e -induced cytotoxicity up to 78% (*Pilecyanidin-e*) but required associating the two polymers in different proportions at a final concentration of 0.738% (*Pilecyanidin-i*) in the culture medium to inhibit up to 82% *MMP-i*.

Looking at the fact that the concentration of *MMP-i* is more pronounced in internal hemorrhoids and *MMP-e* in external hemorrhoids [4,15], *Pilecyanidin-e* was incorporated in a 59.4% VB-Gy solution diluted in water and the liquid was filled in 30 ml sprays for external application; while *Pilecyanidin-i* was mixed in a 96.6% VB-Gy solution to form a gel which was filled in 10 and 50ml tubes, for internal application. A pilot clinical trial was conducted on these preparations to evaluate their efficacy, particularly on external hemorrhoids.

Clinical Trial

Population Distribution

Selected volunteers were randomly assigned to either of the two groups based on their arrival at the clinic, as shown below in the participant flow chart.

Among 71 patients assessed for eligibility, 57 were enrolled in the study with 23 in the placebo and 34 in the Pileseptine active treatment group. 20 patients in the placebo and 31 in the Pileseptine group completed the trial with exploitable results.

Patients of the present study ranged from 12 to 81 years in age, with homogenous distribution in the two groups as given in Table 1.

Table 1: Population analyses with respect to age, sex, location of piles, and type and duration of previous treatment the patients had used

Data	Number of patients in VB-Pile (Pileseptine) group n=31	Percentage of Population	Number of patients in Control (Placebo) group n=20	Percentage of Population
Number of patients	31		20	
Men	16	51.61%	11	55.00%
Women	15	48.39%	9	45.00%
10-35 Age Group	5	16.13%	3	15.00%
36-50 Age Group	17	54.84%	13	65.00%
≥ 51 Age Group	9	29.03%	4	20.00%
Location of Piles				
External	24	77.42%	14	70.00%
Internal	4	12.90%	4	20.00%
Both	3	9.68%	2	10.00%
Presence of Piles				
≤ 12 months	11	35.48%	9	45.00%
≤ 24 months	8	25.81%	6	30.00%
≤ 36 months	7	22.58%	3	15.00%
> 36 months	5	16.13%	2	10.00%
Previous Treatments				
Ayurvedic Remedies	13	41.93%	7	35.00%
Homeopathy	5	16.13%	4	20.00%
Others	11	35.48%	8	40.00%
None	2	6.45%	1	5.00%
Duration of Previous Treatments				
± 1 year	9	29.03%	5	25.00%
± 2 years	7	22.58%	4	20.00%
± 3 years	8	25.81%	5	25.00%
undefined	7	22.58%	6	30.00%

Oedema

As expected, at the beginning of the study, patients in both groups were suffering from severe oedema, with mean scores of 8.42 (±*1.17*) in the VB-Pile group and 7.70 (±*1.46*) in the control group.

After one week of treatment, the oedema severity score showed a sharp decrease in the VB-Pile group with 44% reduction (4.71 ±*1.04*), compared to 13% reduction (6.70 ±*1.46*) in the control group.

In the control group, although the oedema severity score continued diminishing slowly till week 3, with about 30 % reduction compared to pre-treatment, this parameter then remained fairly unchanged in the following weeks, as the mean score only very slightly varied from the 5.45 mark and even showed a slight rise on week 4 (5.75 ±*1.04*).

In the VB-Pile group, however, the marked reduction of oedema observed after only a few days of treatment was further confirmed with around 60% reduction the following two weeks, and 85% reduction of pre-treatment oedema from week 4. The strong and fast effects observed are imputable to the strong hypertonic osmotic activity of the solution, drawing the stagnating hypotonic fluids from the edematous venous plexus, thereby shrinking the swollen tissue back towards its normal size and physiology. The saline solution in the control group had comparatively much lesser osmotic activity, which explains the noticeable but very limited efficacy in abating the oedema.

Pain

The pain sensation was also quite severe in both groups at the beginning of the study, with mean scores of 8.48 (±*1.24*) in the VB-Pile group and 7.95 (±*1.26*) in the control group. After one week of treatment application, the pain was substantially alleviated by 33% in the VB-Pile group, but only slightly (- 4.4 %) in the control group.

In the control group, pain severity showed progressive but moderate amelioration till week 4 with about 33% reduction compared to pre-treatment, the score then remaining stable around 5.3 till the end of the study.

In the VB-Pile group, the pain intensity kept decreasing remarkably each week (- 33% the first week, - 67% after 3 weeks, and - 85% by week 6, compared to the mean severity score at the beginning of the study).

This significant and continuous lessening of the pain sensation in the VB-Pile group is linked to and results from the amelioration of all other symptoms, since as the solution draws liquid from the swollen vessels and oedema is progressively removed, the tissue resumes its normal physiology and the pain recedes. Likewise, as the inflammation regresses, so does the pain associated with it.

Pruritus

Pruritus, or itching sensation, is closely associated with swollen vessels, and is present even in case the hemorrhoids are not especially painful. At the beginning of the study, the mean scores for this parameter are close to the pain scores in each group, respectively.

Whereas the reduction in the pruritus severity progressed only moderately in the control group, with about 11 % reduction after one week, 30% after 3 weeks and 43 % after 6 weeks, in the VB-Pile group the itching sensation is significantly toned down as early as after one week of treatment (- 35%) and continues decreasing markedly throughout the study period (- 74% after 3 weeks, - 90% after 6 weeks), compared to pre-treatment values.

Those results are correlated to the fast shrinking of edematous tissue, and the reduction in hemorrhoid size observed in the VB-Pile group, since when the vascular plexus resumes its normal physiology and functions, this naturally results in relief from pain and itching.

Hydration & Softness

At the study outset, the mean values for hydration or feeling of softness around the hemorrhoids were fairly low in both groups (2.26 in VB-Pile and 2.45 in control groups), as the anal area is irritated and often dry in case of hemorrhoids. In the control group, local anal hydration, compared to the pre-treatment score, barely increased after the first week of treatment (+6%), showing a more noticeable improvement after two weeks (+ 34.4%), but then remaining steady from the 3rd week (around 50% increase, reaching 3.70 on average), indicating that the saline solution is not very effective in providing relief.

In the VB-Pile group, however, a striking intensification of hemorrhoidal hydration could be observed from the 1st week of treatment, with 124% increase. The hydrating effect was not only very strong, but the feeling of softness procured by the treatment proved to be durable, as the score showed a 205% increase by week 2, and continued progressing to 282% by the end of the study (mean: 8.65 ±*1.17*).

The exceptional hydrating activity of VB-Pile results in a soothing effect, directly contributing to the relief from pain and burning sensations on one hand, and also liked to the reduction of oedema on the other hand - 3 parameters which were likewise diminished dramatically from week 1 and throughout the study. This directly demonstrates the efficacy of the first level of the product's mode of action: the anti-edematous & hydrating osmotic activity.

Overall Comfort

As a consistent amelioration is observed in all symptoms associated with hemorrhoids, it is normal that general relief from the condition and enhancement of overall comfort should also be obtained noticeably. In the control group, overall relief and comfort improved modestly but steadily between the first (+ 8%) and third week (+ 87%), when it then plateaued (even marking a slight dip in the last two weeks of treatment).

In contrast, in the VB-Pile group, patient comfort and quality of life improved exceptionally quickly and strongly (+ 100% within only 1 week; and + 300% by the end of the study), demonstrating the superior efficacy of VB-Pile solution in treating all symptoms of hemorrhoids and thus providing significant and effective relief.

Conclusion

The results of this study illustrate some interesting findings, particularly the efficacy of saline solution in providing symptomatic relief against hemorrhoid induced discomforts. The scores of oedema, pain, bleeding, burning sensation, pruritus, and other clinical symptoms, for which the mean severity score was nearly 6.9 at the start of treatment in the placebo group, decreased to 4.35 after 6 weeks of saline application which is very remarkable. These effects were slow and progressive in the placebo group and reached their maximum level within 4 weeks. After 4 weeks of application of the saline solution, although the symptomatic relief was maintained, the efficacy then remained the same up to the end of the study.

In the Pileseptine-treated (VB-Pile) group, the symptomatic relief was extremely fast with the mean pre-treatment symptom score of 7.20 for all parameters rapidly decreasing to 3.65 after 3 weeks and reaching a very minimal level (1.35) after 6 weeks of treatment. The reduction of symptom

severity was very significant: a strong amelioration was observed during the 1st week of treatment, followed by more progressive improvement during the next 3-4 weeks. A remarkable effect was seen on the hemorrhoidal oedema during the 1st week of treatment as the mean severity score was diminished by nearly 50%.

Equally significant results were obtained with the comfort parameter scores where Pileseptine was significantly more effective compared to the saline treatment.

Nearly 50% participants noted some slight local irritation on the hemorrhoidal surface during the 1st 1-2 minutes after spraying Pileseptine. This local irritation was recorded almost throughout the study but was considerably lesser or even absent in patients having minor hemorrhoids and during the second half of the study (after 2-3 weeks). This may have been related to the fact that Pileseptine, being a hypertonic solution, instantly attracts the hypotonic liquid after each application. When piles are dry, the instant liquid exudation thus generated may produce a sensation of irritation.

Among 13 patients who applied the concentrated Pileseptine gel over internal hemorrhoids or lesions, 8 participants mentioned a sensation of warmth and some irritation during the first 5 minutes following each product application. These sensations were temporary and minor, not serious, and were considered to be related to the mode of action of Pileseptine.

It was also noted that no significant variance was observed between the patients having only internal hemorrhoids, those having external hemorrhoids, or those suffering from both, with regards to the evolution in the clinical parameters and results obtained in this study, Pileseptine being found as effective on external as on internal hemorrhoids.

Pileseptine is highly appreciated for the rapidity of results, absence of any major side effects, and for the sensation of freshness over the piles starting 1-2 minutes after each application.

The clinical results were very encouraging as significant improvement was observed in all parameters related to the condition, throughout the treatment course. After 6 weeks of treatment, a clear reduction was observed in hemorrhoidal surface and volume, expansion, prolapse, and severity of all symptoms, with remarkable improvement in overall patient condition and comfort and quality of life of the patients.

Discussion

Hemorrhoids constitute a widespread, worldwide problem, causing considerable physical discomfort and mental distress. In absence of any effective treatment, and because they are located on the intimate areas of the body, hemorrhoids unfortunately remain a very common yet often hidden health problem, as the patients are not readily willing to consult [16].

Hemorrhoids and hemorrhoidal lesions mainly concern deep blood vessels compressed at their neck, leading to the formation of an edematous vascular lesion. As veins are more prone to compression, the return venous flow becomes diminished, and as there is no natural physiological emergency mechanism to release the venous flow, the chronically dilated venous plexus appears as swollen hemorrhoids.

Tissue damage induces an inflammatory response, with an abundance of T-lymphocytes, macrophages, neutrophils, monocytes, mast cells and dendritic cells releasing both anti- and pro-inflammatory cytokines onto the hemorrhoidal surface.

If vascular damage and tissue swelling are not rapidly suppressed, this leads to an inflammatory cascade, the concentration of pro-inflammatory cytokines largely exceeding that of anti-inflammatory cytokines, and the healing process being totally halted [17].

To treat such a pathology, two pre-requisites become naturally evident: first, remove the oedema; and secondly, minimize the concentration of pro-inflammatory cytokines only, if possible.

Unfortunately, we immediately meet two main obstacles: The first one is the absence of any drug or product capable of reducing oedema, as this can only be done by accelerating the venous blood circulation while restricting blood entry into the swollen hemorrhoidal tissue, or by attracting the oedematous liquid out from the swollen vessels without further damaging the tissue and thus exacerbating the inflammatory reaction.

Unfortunately, there is no drug to selectively enhance venous return flow only, and there is no cell-friendly, non-irritant and non-cytotoxic product or method to remove liquid from an oedematous lesion. Currently only variously hypertonic saline solutions are used, but without much success, due to their low osmotic activity.

The discovery of VB-Gy is considered one of the most important steps for the treatment of various topical diseases, as to our knowledge, it is the first, and to this day the only, cell-friendly hypertonic solution, 18 times more osmotically active than sea water, capable of instantly creating a strong outward flow of hypotonic liquid from any semi-permeable live tissue [18].

Being derived from glycerol, VB-Gy is equally a strong natural antiseptic. Furthermore, the filmogen properties of this product facilitate its topical application as a liquid or spray dressing with long-lasting effects.

Application of such a filmogen solution over an oedematous tissue attracts the underlying hypotonic liquid, keeps the tissue or lesion hydrated, reduces the pain and irritation, and helps reduce the concentration of all cytokines present on its surface (both anti- and pro-inflammatory) without any local or systemic toxicity.

Decreasing and removing the hemorrhoidal oedema without harming the tissue is key to the treatment of external as well as internal hemorrhoids. However, this step alone is not sufficient to stop the local inflammatory cascade and prevent reoccurrence of the swelling, as pro-inflammatory cytokines continue damaging the tissue and inducing oedema. Therefore, blocking these cytokines specifically is essential to treating hemorrhoids effectively.

The anti- and pro-inflammatory cytokines present in various topical inflammatory diseases are now relatively well known, and it is also a known fact that while some cytokines are definitively anti- or pro- inflammatory, others can actually become either anti- or pro-inflammatory depending on the location and chronicity of the lesion [19]. The difficulty lies not in identifying the pro-inflammatory cytokines in a lesion but in blocking all these targeted cytokines at once, as no drug, chemical, or biological method can block selectively and simultaneously multiple cytokines.

Although there are no cytokine-inhibiting drugs for the treatment of hemorrhoids, huge efforts have nonetheless been made to block cytokines or cytokine-producing cells for the treatment of psoriatic arthritis, or eczema and dermatitis for instance [20]. Alefacept blocks the activity of target lymphocytes; adalimumab, etanercept, infliximab and golimumab are indicated for inhibiting TNF cytokines while ustekinumab neutralizes the activity if IL-12 and probably IL-23. This proves that although multiple cytokines are involved in a same pathology, a chemical or biological molecule/drug can only block one or maximum two proteins at a time [21]. As it is extremely difficult to launch a new drug containing more than one chemical or biological entity, currently there is no multiple cytokine-inhibiting treatment available for any disease except for the recently launched topical cytokine inhibitor drugs for the treatment of viral diseases, [22,23], chronic wounds and diabetic ulcers [24]. There is presently no anti-oedematous or anti-cytokine treatment for hemorrhoids, and all strategies are directed towards providing symptomatic relief, through cleaning of the hemorrhoids or lesions with saline solutions, using topical or systemic analgesics, anti-inflammatory or vasoconstrictor drugs or calcium antagonists [2,25], and resorting to a surgical approach in severe cases [26]. As the main cause of hemorrhoidal inflammation and oedema is the presence of pro-inflammatory cytokines on the hemorrhoidal surface, we identified these cytokines and used specific polymers as cytokine antagonists to suppress inflammation. To reduce the oedema and simultaneously block all the selected pro-inflammatory cytokines, we incorporated these polymers in the VB-Gy solution thus associating anti-oedema and anti-inflammatory (anti-cytokine) properties in the same product. Polymers are highly branched molecules having a strong affinity for specific macromolecules. In the absence of knowledge regarding the type of cytokines to be blocked, the use of polymeric substances for the treatment of hemorrhoids was not evident, although the use of micronized flavonoids, which are polymeric structures, has been experimented for the treatment of hemorrhoids in recent years [27,28].

The results of this study show that this approach is highly effective not only in reducing hemorrhoidal tissue oedema, but also in remarkably alleviating pain, irritation and itching very quickly compared to the use of saline solution as placebo. It should also be noted that regular and frequent use of physiological saline is also notably beneficial for symptomatic relief but its efficacy reaches a peak level within a few days compared to the Pileseptine treatment where nearly total regression of hemorrhoids was observed within 6 weeks. Unfortunately, the number of patients in the study and particularly the number of subjects suffering from internal hemorrhoids in this study is not sufficient to draw a firm conclusion on the remarkable efficacy of this dual approach treatment but the intensity of results obtained argues in favor of following this hypothesis further.

No side effects were observed in any of the patients, except for the slight and transient feeling of warmth during the 1st few minutes. Investigators suggested using 50 ml tubes for repeated manual application of Pileseptine gel on internal hemorrhoids in order to avoid difficulties in inserting a canula in the anal cavity.

These results open a totally new therapeutic approach of specific cytokine inhibition for the treatment of internal as well as external hemorrhoids. This anti-cytokine theory is already gaining increasing attention and momentum in the research for future drugs [29,30] not only for the treatment of tropical diseases but also cardiovascular pathologies, cancer and arthritis [29-31].

The results of this study show that targeted anti-cytokine approach is not only the most logical, but probably the unique, to date, scientific approach for treating multiple pathologies sorely lacking treatments.

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