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Treatment of gastric ulcers and diarrhea with the Amazonian herbal medicine sangre de grado

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Miller, Mark J. S., Wallace K. MacNaughton, Xiao-Jing Zhang, Jane H. Thompson, Randi M. Charbonnet, Paul Bobrowski, Juan Lao, Ann Marie Trentacosti, and Manuel Sandoval. Treatment of gastric ulcers and diarrhea with the Amazonian herbal medicine sangre de grado. *Am J Physiol Gastrointest Liver Physiol* 279: G192–G200, 2000.—Sangre de grado is an Amazonian herbal medicine used to facilitate the healing of gastric ulcers and to treat gastritis, diarrhea, skin lesions, and insect stings. This study was designed to evaluate the gastrointestinal applications. Gastric ulcers were induced in rats by brief serosal exposure of the fundus to acetic acid (80%). Sangre de grado was administered in drinking water at 1:1,000 and 1:10,000 dilutions from the postoperative period to *day 7*. Guinea pig ileum secretory responses to capsaicin, electrical field stimulation, and the neurokinin-1 (NK-1) agonist [Sar^9,$Met(O_2)^{11}$]substance P were examined in Ussing chambers. Sangre de grado facilitated the healing of experimental gastric ulcer, reducing myeloperoxidase activity, ulcer size, and bacterial content of the ulcer. The expression of proinflammatory genes tumor necrosis factor-α, inducible nitric oxide synthase (iNOS), interleukin (IL)-1β, IL-6, and cyclooxygenase-2 was upregulated by ulcer induction but reduced by sangre de grado treatment, particularly iNOS and IL-6. In Ussing chambers, sangre de grado impaired the secretory response to capsaicin but not to electrical field stimulation or the NK-1 agonist. We conclude that sangre de grado is a potent, cost-effective treatment for gastrointestinal ulcers and distress via antimicrobial, anti-inflammatory, and sensory afferent-dependent actions.

cytokine; inflammation; neuropeptides; *Croton sp.*; complementary medicine

SANGRE DE GRADO (Zangrado), also known as sangre de drago or dragon's blood, is a viscous red tree sap that is used extensively by indigenous cultures of the Amazon River basin for its remarkable healing properties (4, 12, 16). Dragon's blood is a loose term used for any red tree sap and does not always represent this Amazonian herbal medicine, and so we confine our nomenclature to sangre de grado, the name used in Peru, where the material was collected. Applied to the skin for abrasions, cuts, scratches, and blisters, sangre de grado forms a seal, a long-standing barrier, over the lesion (21). This appears to be due to its ability to coprecipitate with proteins or other components of the underlying matrix. For bites and stings in particular, sangre de grado halts the symptoms of pain and itching within minutes, with a subsequent reduction in swelling and redness. The mechanisms for these beneficial effects have remained elusive. Sangre de grado is believed to foster wound healing at a rate that is superior to natural processes and does so with reduced pain, inflammation, and scarring (3, 19, 21), although much of this information is anecdotal.

Sangre de grado's applications are not limited to cutaneous disorders. Sangre de grado is also taken orally, in a dilute form, for severe gastrointestinal distress, e.g., gastritis, gastric ulcer, intestinal infections, and inflammation (4, 12, 19). These applications are the focus of this study. Gastric ulcers and cancer are common afflictions in South America, primarily because of the high incidence of *Helicobacter pylori* infection. In addition, other microbial and parasitic infections are common and place a great burden on gastrointestinal health in the Amazonian communities. Sangre de grado and uña de gato (cat's claw) represent the major ethnomedicines for these complications and remain as such, in view of the high costs of western pharmaceuticals.

Derived from several *Croton* species (*Croton dracanoides, Croton palanostigma, Croton lecheleri*), sangre de grado is easily available throughout the Amazon, with the highest quality material originating in the upper jungle of Peru and Ecuador. The tree is fast growing, reaching heights of 30–45 feet in 3 years. Although the sap can be harvested like rubber (the sap

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flows better in the morning but flows less freely than rubber or maple syrup), repeated tapping of the tree can lead to fungal infections in the tree, thereby diminishing productivity. Current experimental farming techniques are focusing on growing and felling the trees in a 2- to 3-yr cycle. At this time a tree will produce ~1.5 l of sap, a large quantity considering that sangre de grado is applied drop by drop.

The combination of antimicrobial, antioxidant, antiviral, and cicatrizant properties makes sangre de grado a fascinating yet complex herbal remedy for study, one with tremendous opportunities for improvements in health care delivery in the developing world. The experiments detailed in this article are intended to examine the validity of the herbal medicine in its ethnomedical form. We have deliberately attempted not to isolate "single active chemicals" but to evaluate the medicinal as a whole. Our goal was to evaluate and explore the mechanisms underlying the use of this ethnomedicine. By providing this information for the peoples of the Amazon we hope that it will stimulate commercialization of their own natural resources in a sustainable manner, as well as improve local health care delivery in Amazonian communities that cannot afford the luxury of western medicines.

MATERIALS AND METHODS

Gastric ulcer formation. Male Sprague-Dawley rats (200–250 g) were acclimated to the housing facilities for 5 days before initiation of the study. Free access to standard pellet chow was allowed throughout the experimental protocol, with the exception of overnight fasting before induction of the ulcer. All protocols were approved by the Animal Care and Use Committee of the Louisiana State University Medical Center, New Orleans, where the study was conducted.

Ulcers were induced experimentally with acetic acid as we have previously described (5). Briefly, while rats were anesthetized (ketamine, xylazine, and acepromazine) a midline laparotomy incision was performed and the stomach was gently exteriorized. The barrel of a 3-ml syringe, which had been cut, heated, and filed smooth, was placed on the serosal surface of the stomach in the corpus region. Acetic acid (0.5 ml, 80% vol/vol) was instilled into the barrel of the syringe and allowed to remain in contact with the surface of the stomach for 30 s, after which time it was aspirated and the area was rinsed three times with sterile saline. This procedure yields an exposed region of a defined size (60 mm^2) and a corresponding hemispherical ulcer of a comparable size. The stomach was then returned to the abdominal cavity, and the wound was sutured. The rats were then divided into various treatment groups with sangre de grado administered in drinking water. Preliminary studies indicated that sangre de grado had no effect on water intake by rats when delivered in the dilution range of 1:300–1:30,000. The total daily intake of dried sangre de grado latex was 60–600 μg/day in the present study (1:10,000–1:1,000 dilution). After 7 days of treatment the animals were euthanized, and the stomach tissue was collected for determination of ulcer size, myeloperoxidase activity, bacterial content in the ulcer, histopathology, and gene expression.

Bacterial content of ulcers. To determine the bacterial content in gastric ulcers, animals were killed under aseptic conditions. Tissue (~150 mg) was washed in sterile PBS and transferred to sterile, preweighed containers (to determine sample weight), sterile PBS was added, and then the sample was homogenized. Serial dilutions were then plated on MacConkey agar and tryptic soy agar plates and incubated for 18–24 h under aerobic conditions, and the colony-forming units (CFU) were determined using a Leica colony counter. Results are expressed as CFU per gram of tissue (5).

In vitro assessment of epithelial secretion. Experiments were conducted using standard Ussing-type diffusion chambers to determine the effect of sangre de grado on sensory afferent-evoked chloride secretion. Guinea pigs were killed by an overdose of pentobarbital sodium. A segment of ileum was removed 3 cm proximal to the ileocecal junction, flushed with cold (4°C) Krebs buffer and stripped of external muscle. Segments of stripped ileum were opened along the mesenteric border and mounted between two halves of a diffusion chamber, which exposed 0.6 cm^2 of the mucosal surface (Navicyte, San Diego, CA). Ileal segments prepared in this manner retain intact submucosal secretomotor neurons and axon collaterals of primary sensory afferent neurons and respond to capsaicin with chloride secretion (24). Tissues were bathed with Krebs buffer (maintained at 37°C, pH 7.4). Guinea pig experiments were approved by the University of Calgary Animal Care Committee.

The serosal bathing solution contained 10 mM glucose, and the mucosal bathing solution contained 10 mM mannitol. The electrical potential difference across the tissue was maintained at 0 V with a voltage-clamp apparatus (EVC4000, World Precision Instruments, Sarasota, FL). The short-circuit current (I_{sc}) required to clamp the tissue was taken as the measure of active ion transport by the intestinal epithelium and was recorded using a digital data acquisition system (MP-100, BioPac, San Diego, CA). Analysis of recordings was conducted using AcqKnowledge software (version 3, BioPac).

Tissues were allowed to equilibrate with respect to basal I_{sc} and were paired on the basis of basal tissue conductance. Only pairs of tissues with conductances within 20% of each other were included in subsequent experiments. Once a stable baseline was established (~20 min), one member of the pair was exposed on the serosal side to sangre de grado (1:1,000 dilution) while the other member of the pair received the vehicle (1:1,000 dilution of 80% ethanol). Ten minutes later both tissues of the pair were exposed on the serosal side to 100 nM capsaicin. The I_{sc} response was measured and calculated as area under the curve. Once baseline was reestablished, tissues were challenged with electrical field stimulation (EFS) to confirm tissue viability and responsiveness.

To determine the site of action of sangre de grado, experiments were conducted as above with the exception that the neurokinin-1 (NK-1) agonist [Sar^9,$Met(O_2)^{11}$]substance P (Sar-Met-SP, 100 nM; Bachem, Torrance, CA) was added in place of capsaicin. This dose of Sar-Met-SP had previously been shown to stimulate chloride secretion through selective activation of NK-1 receptors (15). The guinea pig ileum was chosen for evaluation specifically because capsaicin is thought to promote secretion only through the release of substance P and the activation of NK-1 receptors (24). Comparing the effects of capsaicin and the NK-1 agonist Sar-Met-SP, we can distinguish between actions of sangre de grado at the level of the sensory nerve itself vs. an effect on substance P-dependent processes. EFS was used to evaluate any nonspecific effect on cholinergic nerves or the secretory apparatus itself.

Gene expression. RNA was extracted from frozen rat stomach by the guanidine thiocyanate extraction method immediately after tissue collection (22). First-strand cDNAs were synthesized from 0.5 μg of total RNA using oligo(dT) and

Table 1. *Primer sequences*

COX-1 rat (U03388) 457 bp		
Forward 5′ TCT GCC TCA ACA CCA AGA CC 3′	20 bp	1761–1780
Reverse 5′ ATT CAA CTG CCT CTG CCT TC 3′	20 bp	2217–2198
COX-2 rat (S67722) 687 bp		
Forward 5′ TCC AAA CCA GCA GGC TCA TAC 3′	21 bp	1068–1088
Reverse 5′ TCC TAA ACC CCA CTT CTC CTC C 3′	22 bp	1754–1733
IL-1β rat (E05490) 491 bp		
Forward 5′ TGT CCA GAT GAG AGC ATC CAG C 3′	22 bp	189–210
Reverse 5′ TGT TTG GGA TCC ACA CTC TCC A 3′	22 bp	679–659
IL-6 rat (M26744) 353 bp		
Forward 5′ CTC TCC GCA AGA GAC TTC CAG C 3′	21 bp	74–95
Reverse 5′ CGG AAC TCC AGA AGA CCA GAG C 3′	21 bp	426–405
TNF-α rat (X66539) 295 bp		
Forward 5′ TAC TGA ACT TCG GGG TGA TCG GTC C 3′	25 bp	149–173
Reverse 5′ CAG CCT TGT CCC TTG AAG AGA ACC 3′	24 bp	443–420
Inducible NOS human, murine, and rat (D14051) 907 bp		
Forward 5′ TCG AAA CAA CAG GAA CCT ACC A 3′	22 bp	529–550
Reverse 5′ ACR GGG GTG ATG CTC CCG GAC A	22 bp	1435–1414

COX, cyclooxygenase; IL, interleukin; TNF-α, tumor necrosis factor-α; NOS, nitric oxide synthase.

SuperScript II Reverse Transcriptase System (GIBCO BRL, Grand Island, NY). First-strand cDNA templates were amplified by the polymerase chain for glyceraldehyde-3-phosphate dehydrogenase, inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-1, COX-2, interleukin (IL)-1β, and IL-6 using a hot start and *Taq* polymerase (Ampliwax and Amplitaq, Perkin-Elmer, Foster City, CA). Denaturation, annealing, and elongation temperatures for iNOS were as follows: 95°C for 3 min, followed by 94°C for 30 s, 60°C for 45 s, and 72°C, for 1.5 min, each for 30 cycles, with a final cycle of 72°C for 4 min. The negative control was from a cDNA reaction that used water in place of RNA. Oligonucleotide primers were based on the sequence of a conserved region of mouse and human iNOS. PCR products were separated in a 2% agarose gel and visualized by ethidium bromide staining. Gels were visualized under ultraviolet (UV) light and photographed using a Kodak Electrophoresis Documentation and Analysis system 120 (Eastman Kodak, Rochester, NY). Essentially similar techniques with appropriate adjustments in cycles and annealing and elongation temperatures were used for the other transcription-regulated products evaluated in this proposal. Details of the sense and antisense primer sequences for these gene products are given in Table 1.

Materials. Sangre de grado (Zangrado) was supplied by Rainforest Phytoceuticals (Delmar, NY) in its pure form. Zangrado was collected from the highland jungle of the Huallaga River Valley in tropical Peru (a tributary of the Amazon River) and verified by Eng. Warren Rios (Universidad Nacional Agraria de al Selva, Tingo Maria, Peru). For the current purposes, the sangre de grado that was used was a mixture of the subspecies *Croton lecheleri* and *Croton palanostigma*, as wild-harvested sangre de grado was obtained from indigenous peoples and both of these subspecies grow in the Upper Huallaga valley of Peru. We are not aware of any difference in bioactivity attributed to these subspecies at this time. All other materials were of research grade and were obtained from Sigma Chemical (St. Louis, MO), except as noted.

RESULTS

Gastric ulcers. The acetic acid model of gastric ulceration produces an ulcer of reliable, reproducible dimensions that persists for 7 days followed by gradual healing (5). We chose a treatment schedule from *day 0* to *day 7* to determine whether sangre de grado promotes ulcer healing, and if so, to identify the underlying mechanisms. Ulcer size (length × thickness, determined histologically) was reduced by sangre de grado at both 1:1,000 and 1:10,000 dilutions ($P < 0.05$, Fig. 1). This healing was of similar magnitude to what we had described with a combination of penicillin and streptomycin (5, 6). Related to this antibacterial therapeutic action, we noted that the bacterial content of the ulcer was greatly reduced by sangre de grado treatment at both concentrations ($P < 0.01$, Fig. 2).

In contrast to the results of our previous studies with antibiotics and probiotics (5), sangre de grado also reduced the granulocyte content of gastric ulcers (Fig. 3, $P < 0.05$). Thus experimental ulcers were smaller and less inflamed and had reduced bacterial colonization. Examples of this healing are shown in Fig. 4. The ability of sangre de grado to kill bacteria in vitro has already been described (3) and was confirmed in this in vivo model. However, we readdressed the antibacterial

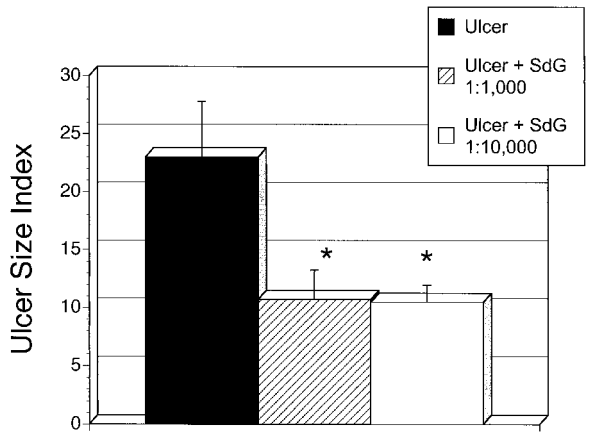

Fig. 1. Alterations in gastric ulcer size (as determined by length and depth) with sangre de grado (SdG) treatment. Control ulcers (solid bar) were significantly larger (*$P < 0.05$) than in rats treated with sangre de grado at either 1:1,000 or 1:10,000 dilutions administered via drinking water. All groups were evaluated 7 days after ulcer induction.

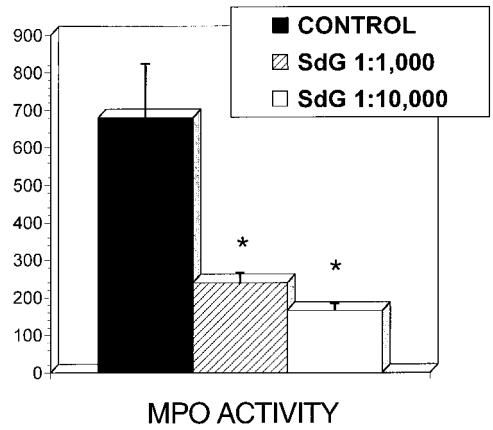

Fig. 2. Myeloperoxidase (MPO) activity in ulcers of rats with experimental gastric ulcers. Treatment with sangre de grado in drinking water at dilutions of 1:1,000 or 1:10,000 significantly reduced ulcer MPO levels (*$P < 0.05$). All groups were evaluated 7 days after ulcer induction.

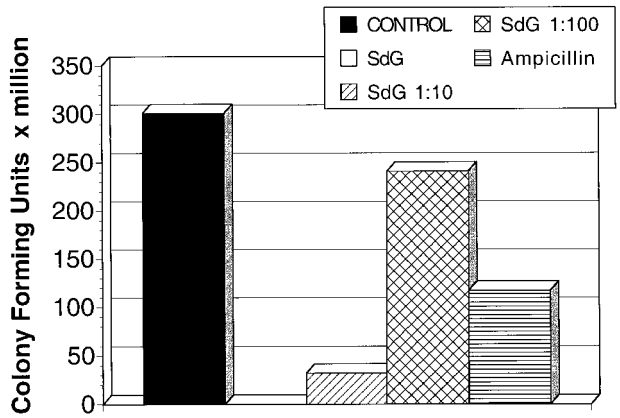

Fig. 4. The antibacterial actions of sangre de grado in vitro. Different concentrations of sangre de grado were streaked onto plates, allowed to dry, and then treated with *E. coli*. Bacterial colonies were absent in plates treated with pure sangre de grado. With dilution, the antibacterial effects of sangre de grado were reduced, with the 1:100 dilution being indistinguishable from control. The effects of ampicillin are included for comparative purposes.

potency of sangre de grado in vitro using *Escherichia coli*. Undiluted sangre de grado was completely effective in killing *E. coli.* At 1:10 dilution sangre de grado was still 90% effective, but at 1:100 dilution its ability to reduce CFU was indistinguishable from control values. These results are depicted in Fig. 5. with ampicillin noted as a positive control.

On histopathological examination, the untreated ulcers displayed profound necrosis of the gastric epithelia with cellular debris mixed with bacteria (Fig. 6). In rats that received sangre de grado in drinking water, areas of ulceration remained but regions of regenerating epithelia were evident. The mucosa was still inflamed, but it was clear that the healing process had been initiated (Fig. 6).

Gastric ulceration establishes a local inflammatory response that may also retard the healing process and is driven by the exposure of the mucosa to gastric contents after disruption of the epithelial barrier and colonization of the ulcer bed. To characterize this inflammatory response we collected tissue for RT-PCR. Figure 7 depicts the RT-PCR results of inflammatory gene expression in normal rats and rats with gastric ulcers with and without sangre de grado treatment. COX-1 was the only gene product evident in normal rats. Furthermore, the expression of COX-1 was not affected by the induction of gastric ulceration, as expected. COX-1 expression also remained steady with sangre de grado treatment (Fig. 7). On the other hand, the expression of iNOS, tumor necrosis factor-α (TNF-α), IL-1β, and IL-6 was minimal or absent in normal rats and markedly upregulated in gastric ulceration. Treatment with sangre de grado reduced the expression of these genes, in particular iNOS and IL-6 and, to a lesser extent, TNF-α, COX-2, and IL-1β.

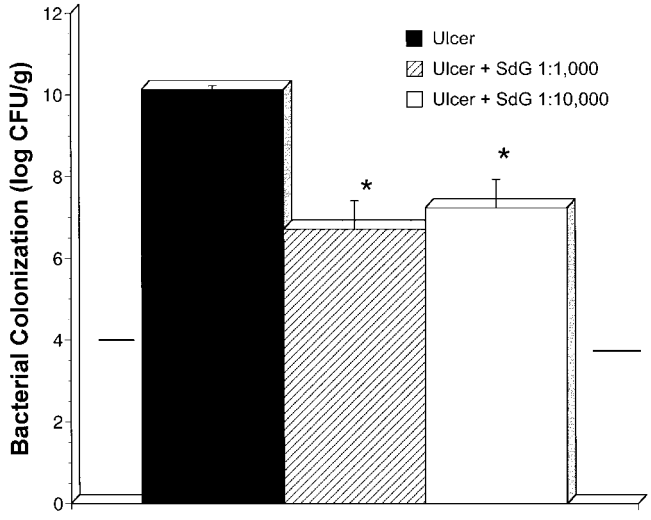

Fig. 3. Levels of bacteria in the ulcer bed, as denoted by colony forming units (CFU). Nontreated ulcers had significantly more bacteria than those treated with diluted sangre de grado (1:1,000 or 1:10,000, *$P < 0.05$) administered via drinking water. The horizontal line depicts the bacterial content of normal rat stomach.

Intestinal epithelial secretion. The ability of sangre de grado to provide relief from cutaneous stings and bites as well as a therapy for diarrhea may be caused by an action on sensory afferent neurons (10). To determine whether sangre de grado acted on sensory afferent nerves, we used a previously described bioassay of capsaicin-evoked chloride secretion from isolated segments of guinea pig ileum mounted in Ussing chambers (24). Exposure to capsaicin caused an immediate increase in I_{sc} indicative of chloride secretion. The peak response occurred within 5 min and returned to baseline within 10–15 min. The other agonists used to promote chloride secretion, EFS and Sar-Met-SP, produced qualitatively similar changes in I_{sc} to capsaicin. Pretreatment with serosal sangre de grado, at a dilution of 1:1,000, was noted to selectively attenuate by ~70% the secretory responses to capsaicin (Fig. 8, $P < 0.01$), quantified as a change in I_{sc} (μA/cm^2). In contrast, the I_{sc} responses to EFS and the NK-1 agonist Sar-Met-SP were unaffected by sangre de grado. The I_{sc} response to capsaicin in this preparation is medi-

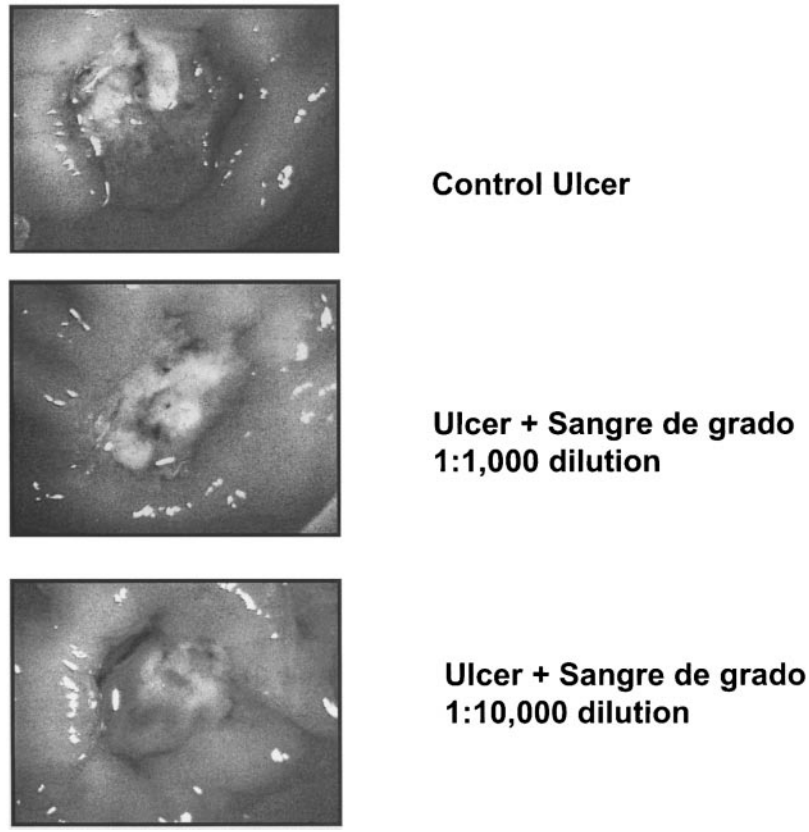

Fig. 5. Gross morphology of experimental gastric ulcers. In the top panel from control rats, a round ulcer is clear. In the bottom two panels are examples of rats treated with sangre de grado in drinking water at dilutions of 1:1,000 and 1:10,000, respectively. In sangre de grado-treated rats, the smaller ulcers are evidence of healing.

ated by the release of substance P from sensory afferents with a subsequent activation of NK-1 receptors (15, 24). Thus the selective effect on capsaicin-evoked responses suggests that suppression of epithelial secretion was caused by a direct effect on the sensory afferents and not by an action on the neurotransmitter, its receptor, or its cellular target.

DISCUSSION

Ethnomedicines like sangre de grado are the mainstay of health care delivery in tropical South America, in part because of their rich history and confidence in their use but also because western pharmaceuticals are beyond the financial reach of these communities. This is not a unique problem; similar hurdles face most of the developing world. Although the use of traditional medicines may be widespread, there is little direct evidence as to the efficacy of these therapeutic approaches, and this lack of data is particularly apparent for Amazonian medicinals. Certainly, pharmacognosy studies have been performed (19) with a number of unique chemicals isolated from sangre de grado. However, we are left with an informational void as to how these phytoceuticals work, the breadth of their applications, and how they can be combined with other therapeutic approaches (traditional or pharmaceutical). This information is required not only for the peoples of tropical South America but also for the public of western nations who are embracing herbal medicines as a component of their own health maintenance.

Sangre de grado is widely available in Amazonia, where it is used for a large variety of conditions—for insect bites and stings, for wound healing, to limit the blood loss in childbirth, and, relevant to this investigation, to heal gastric ulcers and for diarrhea (4, 12, 16). In this study we treated experimental gastric ulcers in rats with sangre de grado in a fashion that was consistent with ethnomedical traditions. Sangre de grado is taken orally in a highly diluted form, e.g., three drops in a beverage two or three times a day or, alternatively, a teaspoon in a liter of water with a cup of this stock drunk two or three times a day. We duplicated this approach by introducing sangre de grado in the drinking water of rats at dilutions of 1:1,000 or 1:10,000. At these concentrations, sangre de grado was particularly effective in healing gastric ulcers, with an estimated intake of dry sangre de grado latex of 60–600 $\mu g \cdot rat^{-1} \cdot day^{-1}$.

The mechanisms for ulcer healing appear to involve the antimicrobial effects of sangre de grado. Gastric ulcer healing is determined by the bacterial content of the ulcer bed as we have previously described (5). Antibiotics or the promotion of lactobacillus colonization lead to ulcer healing. In this study, sangre de grado had similar effects. Sangre de grado markedly reduced the bacterial content of the ulcer concomitant with healing and reduced inflammation. A causal link between reduced bacterial load and sangre de grado is likely, because in vitro sangre de grado was a highly effective antibacterial agent (Fig. 5). However, sangre

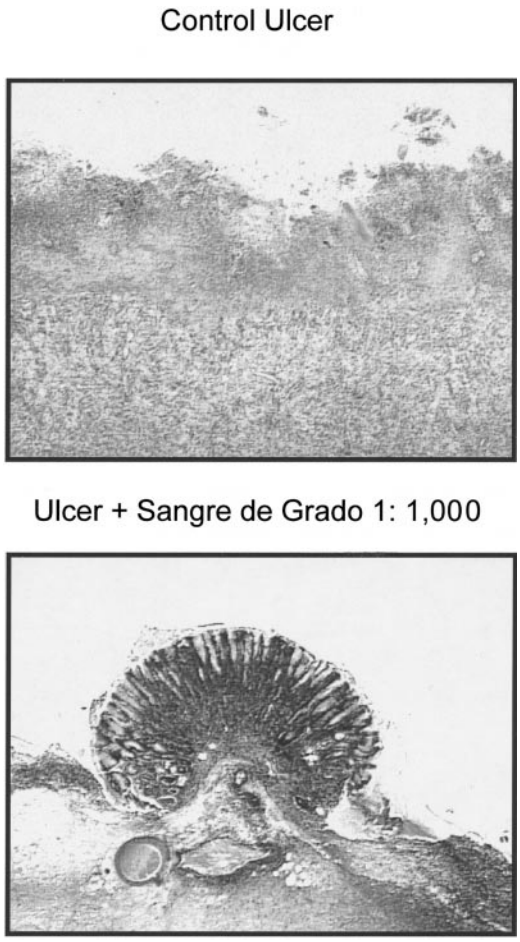

Fig. 6. Low-power photographs (×40) of hematoxylin and eosin-stained sections of experimental gastric ulcers. Note that in the control, untreated ulcer (*top*) the mucosa has been lost and replaced by a layer of necrotic tissue with an underlying region of muscularis that has a heavy leukocyte infiltration. An example of an ulcer treated with sangre de grado, in which regeneration of the mucosa and epithelialization are apparent, is shown at *bottom*.

de grado was administered to the rats in a highly dilute form, and in vitro the bactericidal activities were evident at higher concentrations. The reasons for this discrepancy are unclear. It appears that sangre de grado makes the microenvironment either unsuitable for bacterial growth or more susceptible to other means of clearance, as has been suggested for epidermal growth factor (EGF) (6). When sangre de grado is mixed with rat gastric mucus there is a change in UV absorbance, suggesting an interaction that may alter the suitability of this microenvironment for colonization (data not shown).

The healing effects of sangre de grado on ulcer size and bacterial numbers rivaled the combination of penicillin and streptomycin (5) as well as EGF (6, 14), but, in addition, sangre de grado also reduced the mucosal inflammatory response, an effect not seen with antibiotics. Myeloperoxidase (MPO) activity is used as an index of granulocyte content/infiltration in tissues, and ulcer MPO levels were greatly reduced by sangre de grado treatment. In our previous study, MPO values

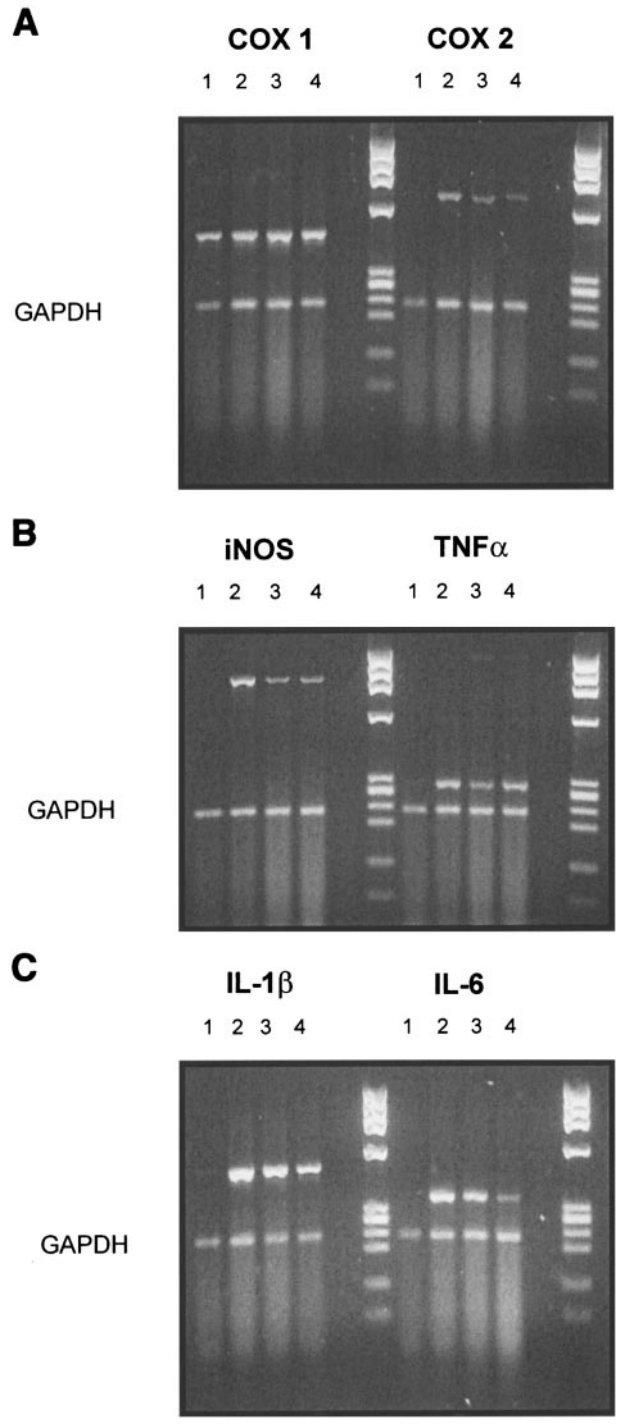

Fig. 7. Gel depicting RT-PCR products for cyclooxygenase (COX)-1, COX-2, inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 from rat stomach. *Lane 1* is derived from an ulcer-free naïve rat; *lane 2* is from a control, untreated ulcer; *lane 3* is from an ulcer treated with sangre de grado (1:1,000 dilution); *lane 4* is from an ulcer treated with sangre de grado (1:10,000 dilution). *A*: COX-1 (*left*) and COX-2 (*right*). *B*: iNOS (*left*) and TNF-α (*right*). *C*: IL-1β (*left*) and IL-6 (*right*). Note that COX-1 expression was consistent in all groups. In contrast, COX-2, iNOS, TNF-α, IL-1β, and IL-6 were absent in normal animals and elevated in ulcer-bearing stomachs but reduced by sangre de grado treatment. GAPDH, glyceraldehyde-3-phosphate dehyrogenase.

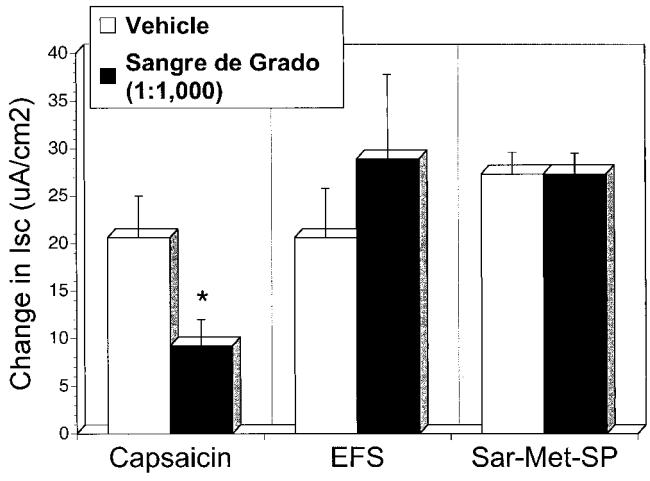

Fig. 8. Epithelial secretion as determined by changes in short-circuit current (I_{sc}, $\mu A/cm^2$) in guinea pig ileum. Secretion was induced by capsaicin, electrical field stimulation (EFS), and the neurokinin-1 agonist [Sar^9,$Met(O_2)^{11}$]-substance P (Sar-Met-SP). Serosal application of sangre de grado (1:1,000 dilution) reduced by ~70% the secretory response to capsaicin (*$P < 0.05$), whereas the responses to NK-1 activation or EFS were not significantly altered.

were unaffected by antibiotic administration. This suggests that sangre de grado may promote ulcer healing through additional mechanisms, and these may involve a direct effect on the inflammatory response. Inhibition of neurogenic inflammation, as indicated by the ability of sangre de grado to inhibit the activation of sensory afferent nerves, is a possible explanation.

Expression of proinflammatory genes was used to evaluate ulcer-induced gastritis and the anti-inflammatory effects of sangre de grado. In normal rat stomach COX-1 was expressed, whereas the genes for TNF-α, iNOS, IL-1β, IL-6, and COX-2 remained in their quiescent state. Induction of gastric ulcers resulted in the expression of these latter proinflammatory genes, whereas the expression of COX-1 remained unaltered. Sangre de grado suppressed the activation of these proinflammatory genes, particularly iNOS. This effect may be due, in part, to a reduction in the bacterial content of the ulcer, thereby reducing the signals activating the transcription of these gene products. The reduced infiltrate of inflammatory cells may also contribute to the reduced expression of these proinflammatory genes in the ulcer tissue. Together, these results support an action of sangre de grado to promote ulcer healing, as indicated by Amazonian shamans.

The ethnomedical use of sangre de grado for intestinal distress and diarrhea was evaluated in vitro for a potential suppression of secretagogue responses. Sangre de grado had no effect on EFS- or NK-1-induced secretion. This suggests that it does not compromise submucosal secretor motor neuron-induced epithelial secretion, cholinergic or substance P-dependent processes. On the other hand, the responses to capsaicin were greatly attenuated. In the guinea pig ileum preparation this indicates that sangre de grado directly compromised sensory afferent activation. Vanner and MacNaughton (24) showed that acute application of capsaicin to these preparations causes chloride secretion that is entirely dependent on the activation of extrinsic sensory afferent neurons. The response to capsaicin was abolished by pretreatment with tetrodotoxin, confirming the neural dependence of the response. Secretory responses to capsaicin in this preparation are caused by the activation of NK-1 receptors (15, 24), and to address the potential interaction at the NK-1 receptor level we evaluated the selective NK-1 agonist Sar-Met-SP. Epithelial secretory response to NK-1 activation was not altered by sangre de grado. Another control was EFS; EFS depolarizes all neurons in the preparation to elicit a net chloride secretion (2). Sangre de grado did not alter the secretory response to EFS, indicating that it did not act as a general inhibitor of nerve activation. Not shown were the secretory responses to carbachol, which directly activates epithelial cells to promote secretion; responses to carbachol were also unaltered by sangre de grado.

Thus it appears that the ethnomedical reports of sangre de grado's utility in the treatment of diarrhea and intestinal distress involve a selective suppression of nonmyelinated sensory nerves and thereby neuropeptide-dependent responses. This is a unique therapeutic approach. Sangre de grado therapy should not only reduce the secretory response (as quantified in this study) but also other sensory afferent-dependent mechanisms—cramping and pain perception. These data offer compelling evidence to support the evaluation of sangre de grado for therapeutic utility in other states of neurogenic inflammation.

An extract of sangre de grado, SP-303, consisting of proanthocyanidins, has been shown to block intestinal secretory responses via cAMP-mediated mechanisms (2), including cholera toxin. An additional study in AIDS-associated diarrhea indicated that sangre de grado reduces stool weight and frequency (8). The doses of SP-303 required to suppress intestinal secretion appear to be higher than those described for sangre de grado in its pure ethnomedical form. This leads us to conclude that proanthocyanidins are not the only chemical constituents responsible for the efficacy of sangre de grado in treating diarrhea. We are unaware of any reports of effects of SP-303 on sensory afferents. However, we postulate that the ability of sangre de grado to block capsaicin-activated sensory afferents may indicate that it attenuates the cramping and pain associated with intestinal distress as well as secretory processes. Sensory afferent activation has long been associated with intestinal secretory responses to a diversity of stimuli. More recently, rotavirus-induced secretory diarrhea has been linked to the enteric nervous system (13). Lundgren et al. (13) postulated that agents that inhibit neurally driven intestinal secretion may offer a means of limiting secretory diarrhea in response to numerous initiators. It appears unlikely that cholinergic antagonists with sufficient selectivity will be available to achieve that goal, but sangre de grado with its selectivity for sensory afferents could be the prototypical therapeutic agent. Its low cost and the

high incidence of diarrhea in the developing world make its applications quite attractive.

Although the inhibition of sensory afferent mechanisms may explain the utility of sangre de grado in diarrhea and intestinal cramping, it is less clear whether this mechanism is involved in ulcer healing. Neuropeptides (mainly calcitonin gene-related peptide) are protective of the gastric mucosa under acute protocols (9). In models of chronic colitis, neurokinin receptor antagonism failed to promote healing (25). Thus the importance of suppression of sensory afferent activation in ulcer healing remains unclear. However, this mechanism is a likely explanation for the ethnomedical use of sangre de grado for the treatment of insect bites, stings, and burns. Suppression of sensory afferent activation would block the pain and symptoms associated with these conditions. Ulcer healing in this model has been achieved by either antibiotic administration (5, 6) or EGF (6). Reductions in EGF from salivary glands by cigarette smoking retards ulcer healing (14). However, neither antibiotics nor EGF reduces the MPO activity of the ulcer bed despite reductions in the bacterial load. In contrast, sangre de grado does reduce ulcer MPO activity. The reasons for additional anti-inflammatory action of sangre de grado is not clear, but it is interesting to speculate that it may result from an inhibition of neurogenic inflammation.

Ulcer healing by sangre de grado may also be due to other chemical constituents. Although proanthocyanidins are the major chemical class present in sangre de grado, there are a number of other chemicals that have been isolated and are thought to be involved in the diverse effects exhibited by sangre de grado. Crolechinol, crolechinic acid, korberin A and B, 3′,4-*O*-dimethylcedrusin, and taspine (3, 19) have received considerable attention, although only a few studies have evaluated this herbal medicine. 3′,4-*O*-dimethylcedrusin and the polyphenolic fraction have been suggested to be the chemicals responsible for wound healing via fibroblast activation (1, 3, 23). Taspine is present in Peruvian sap but is less evident in Ecuadorian sap and has been implicated in its use in inflammation and cancer because it readily kills tumor cells (3, 20). In studies in cell culture, sangre de grado inhibits cell proliferation yet protects against cell death initiated by media starvation (11, 18, 20). This suggests a critical action at the level of cell cycle regulation and apoptosis, which we have explored. Taspine has been touted as a principal component of the wound healing actions of sangre de grado, on the basis of its early stimulation of wound repair (3, 11, 23). However, the taspine content varies with geographical location, although the ethnomedical use of sangre de grado for wound repair is widespread throughout Amazonia, leading many to contemplate that other chemicals are important, including the polyphenols (1). The efficacy of sangre de grado, including the cicatrizant effect, is perhaps better explained by the array of chemicals acting in concert rather than a single chemical. For example, beyond the antiviral actions of proanthocyanidins, antimicrobial actions may be critical, an effect thought to be due to 1,3,5-trimethoxybenzene and 2,4,6-trimethoxyphenol, which are present in trace amounts but are 30 times more potent than penicillin (3, 19).

In summary, sangre de grado is an effective treatment for the healing of gastric ulcers well as an antidiarrheal agent. The ability of sangre de grado to reduce intestinal fluid secretion appears to be due to its ability to selectively inhibit epithelial electrolyte movement driven by sensory afferent nerves and not to a direct effect on the secretory apparatus at the doses tested. In terms of ulcer healing, its effectiveness was equivalent to either antibiotics or EGF, with the added benefit of reducing the degree of granulocyte infiltration. With further research the potential of this medicinal plant in managing gastrointestinal disease will be placed in proper perspective, but its low cost, effectiveness, and wide clinical experience in Amazonia are encouraging.

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