Inhibition of Neurogenic Inflammation by the Amazonian Herbal Medicine Sangre de Grado

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This study was designed to determine if the Amazonian medicinal sangre de grado, confers benefit by suppressing the activation of sensory afferent nerves. Methods: (i) vasorelaxation of rat mesenteric arteries in response to calcitonin gene-related peptide; (ii) rat paw edema in response to protease-activating peptide receptor 2–activating peptide; (iii) rat paw hyperalgesia in response to low-dose protease-activating peptide receptor 2–activating peptide or prostaglandin E_2; (iv) gastric hyperemia in response luminal capsaicin; (v) a clinical trial of a sangre de grado balm in pest control workers. The parent botanical was fractionated for evaluation of potential active components. In preconstricted rat mesenteric arteries, highly diluted sangre de grado (1:10,000) caused a shift to the right of the calcitonin gene-related peptide dose–response curve ($p < 0.01$). Paw edema in response to protease-activating peptide receptor 2–activating peptide (500 μg) was reduced by as single topical administration sangre de grado balm (1% concentration, $p < 0.01$) for at least 6 h. Hyperalgesia induced by either low-dose protease-activating peptide receptor 2–activating peptide (50 μg) or prostaglandin E_2 was prevented by sangre de grado balm. A fraction possessing analgesic and capsaicin antagonistic properties was isolated and high-performance liquid chromatography and gas chromatography–mass spectrometry analysis indicated that it was a proanthocyandin oligomer. In pest control workers, sangre de grado balm (Zangrado) was preferred over placebo, for the relief of itching, pain, discomfort, edema, and redness in response to wasps, fire ants, mosquitoes, bees, cuts, abrasions, and plant reactions. Subjects reported relief within minutes. We conclude that sangre de grado is a potent inhibitor of sensory afferent nerve mechanisms and supports its ethnomedical use for disorders characterized by neurogenic inflammation. ***Key words: capsaicin/Croton lechleri/itch, pain/sensory afferent nerves. J Invest Dermatol 117:725–730, 2001***

Sangre de grado (SdG), 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 (Duke and Vasquez, 1994; Jones, 1995; Phillipson, 1995). Derived from several Croton species (*C. dracanoides*, *C. palanostigma*, *C. lechleri*), SdG is common throughout Amazonia, with the highest quality originating in the upper jungle of Peru and Ecuador. The tree is fast growing, reaching heights of 30–45 ft (10–15 m) in 3 y. Whereas the sap can be harvested like rubber, trees that are repeatedly tapped are prone to fungal infections, thereby diminishing productivity. Current experimental farming techniques focus on growing and felling the trees in a 2–3 y cycle; at this time a single tree will produce approximately 1.5 l of sap.

Manuscript received December 19, 1999; revised May 7, 2001; accepted for publication May 22, 2001.

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Abbreviations: protease activated receptor-2 activating peptide, PAR-$_2$AP; calcitonin gene-related peptide, CGRP; sangre de grado, SdG; vanilloid receptor, VR.

Mechanistically, little is known about SdG given the region and cultures from which it originates. Applied to the skin for abrasions, cuts, scratches, blisters, bites, and stings, SdG forms a long-standing barrier possibly due to its ability to coprecipitate with proteins or other matrix elements (Uchida *et al*, 1990). In so doing it is claimed to foster accelerated wound healing and does so with reduced pain, inflammation, and scarring (Vaisberg *et al*, 1989; Porras-Reyes *et al*, 1993; Chen *et al*, 1994).

SdG's applications in Amazonia are not limited to cutaneous disorders. SdG is also taken orally, in dilute form, for severe gastrointestinal distress, e.g., gastritis, gastric ulcer, intestinal infections, and inflammation (Maxwell, 1990; Duke and Vasquez, 1994; Jones, 1995). In addition, because SdG has hemostatic properties it is sometimes used to arrest excessive bleeding associated with childbirth (this hemostatic effect is also useful for severe cuts and lacerations). As this information is largely anecdotal, the purpose of the present investigation was to evaluate the mechanisms underlying the proposed efficacy of SdG in a scientifically rigorous manner.

MATERIALS AND METHODS

Vasorelaxation of rat mesenteric arteries Mesenteric arteries were removed from anesthetized 3 mo old female Fisher 344 rats (National

Institutes of Aging) for the study of diameter changes, as previously described by us (White *et al*, 1996). The small intestine was excised immediately after sacrifice of the rat and placed in ice-cold bicarbonate buffer solution of the following composition (in mm): NaCl, 130; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 14.9; dextrose, 5.5, $NaCa_2EDTA$, 0.03; $CaCl_2 \cdot 2H_2O$, 1.6. Resistance-size arteries (3rd–4th order branches of the superior mesenteric artery; approximately 250 μm outer diameter) were cleaned of adherent fat and connective tissue and cannulated at each end with glass microcannulae in an arteriograph chamber. These arteries are located at the margin of the intestine, just before the arteries branch and actually enter the intestinal wall. The arteries were pressurized and maintained at 60 mmHg with a servo-control device. Warm buffer (37°C) bubbled with a mixture of 95% O_2, 5% CO_2 was circulated through the arteriograph chamber at a rate of 20 ml per min.

All drugs and mediators were added to the superfusion solution. The arteriograph was placed on the stage of an inverted microscope, and the image of the artery projected on to a video monitor. The image of the artery was continuously scanned and measurements of the outer diameter stored in a computer file for analysis. Arteries were allowed to equilibrate at 60 mmHg, in warmed, circulating buffer for a minimum of 45 min prior to the initiation of the experimental protocol.

Arteries were constricted with an EC_{80} concentration of phenylephrine (the concentration needed to produce 80% of the maximal contractile response), and calcitonin gene-related peptide (CGRP) was added to the superfusate bath at increasing concentrations until a full relaxation response was achieved. The superfusate was washed, re-equilibrated and phenylephrine was administered to raise vascular tone. SdG (1:10,000 dilution) was added to the bath and the dose–response curve to CGRP repeated. In a separate study, the CGRP alone administration was repeated without coadministration of SdG. At the conclusion of the experiment, the bath was rinsed, the vessel preconstricted and the vasodilator response to acetylcholine evaluated as an example of a sensory afferent independent vasodilator. Relaxations of arterial segments to CGRP are expressed as a percentage of the phenylephrine-constricted diameter. Data were analyzed using a nonlinear regression of sigmoidal dose–response curves (using GraphPad Prism 2.01) from which an EC_{50} value (concentration of CGRP that elicited 50% of the maximum response), maximum response and slope. The negative logEC_{50} values (pD2) in the absence and presence of SdG (1:10 000 dilution) were compared using a paired t test (Statistica, Statsoft).

Rat paw edema Sprague–Dawley rats (250 g) were anesthetized with sodium pentobarbitol. Twenty minutes prior to the intradermal injection of protease activated receptor-2 activating peptide (PAR_2-AP) (SLIGRL-NH2, 500 μg) into the rat footpad, rats received either 40 mg of placebo balm or balm containing SdG (1%, Zangrado Bug Bite Balm, Rainforest Phytoceuticals, LLC, Delmar, NY, http://www.amazonmedicines.com) topically to the footpad. This dose of PAR_2-AP was chosen as based on our previous experience that it elicits paw edema through neurogenic mechanisms (Steinhoff *et al*, 2000). Basal paw volume was measured before PAR_2-AP administration, and every subsequent hour for a total of 6 h, as previously described (Vergnolle *et al*, 1999; Steinhoff *et al*, 2000). Paw volume was measured using a hydroplethysmometer bath (Ugo Basile, Italy) by an individual unaware of the treatment group.

Rat paw hyperalgesia

Induced by PAR_2-AP Rats were treated in a manner similar to that described above for paw edema studies except that the dose of PAR_2-AP was reduced to 50 μg (intradermal injection) to avoid complications associated with edema formation. Rats were divided into two treatment groups: (1) control, who received the placebo balm (devoid of SdG), and (2) Zangrado balm (1% SdG). Both were delivered topically (40 mg) 15 min before intradermal administration of PAR_2-AP. Paw withdrawal latency time to a thermal stimulus, as determined in a Hargreave's apparatus, was used as the index of pain sensitivity. This involves the measurement of the time it takes for a rat to move (withdraw) its foot away from a thermal stimulus (Vergnolle *et al*, 1999). Withdrawal times were determined in each group prior to PAR_2-AP administration (basal), and then 30 and 60 min after PAR_2-AP administration. A reduction in latency withdrawal time is used as an index of hyperalgesia.

Induced by prostaglandin (PG) E_2 Similarly to the experiments where hyperalgesia was induced by PAR_2-AP, paw hyperalgesia was also induced by PGE_2 (0.3 μg), administered by intradermal injection. Basal withdrawal times were established and either placebo or Zangrado balm containing 1% SdG were applied topically to the paw. Withdrawal times were determined every 15 min over the course of 1 h, at which time the PGE_2 hyperalgesia response had returned to baseline.

Rat gastric hyperemia in response to capsaicin As previously outlined (Gronbech and Lacy, 1996), gastric blood flow was measured by a laser Doppler flow meter placed on the gastric luminal surface of anesthetized, laparotomized rats. This involves the use of a plexiglass chamber used for isolating the gastric mucosal surface whereas the stomach remains fully innervated and with its blood supply intact. The luminal surface is continually bathed in a buffered salt solution to which capsaicin (320 μm), alone or with pretreatment with SdG (1% solution) or SdG fractions. Hyperemia is expressed as a percentage change from baseline.

SdG fractionation and structural analysis The SdG latex (30 ml) was mixed with 270 ml of acetate buffer (1 mm sodium acetate, 1 mm acetic acid, pH 4), vortexed and centrifuged at $1200 \times g$ for 3 min After removal of the supernatant the precipitant was then mixed with methanol (30 ml), vortexed, and centrifuged at $1200 \times g$ for 3 min. The supernatant was applied to a C18 sephadex cartridge. Eluants were collected with various water–methanol (0, 20, 40, 60, 80, and 100%) washes and dried for analysis of biologic properties. Similarly, the supernatant (20 μl) was also subjected to high pressure liquid chromatography analysis. After filtration in a 20 μm filter, the supernatant components were separated using a Varian Dynamax high pressure liquid chromatography system equipped with two Model SD-200 pumps, and a model 330 diode array detector, and a microsorb-MV C-18 reverse phase column (25×0.46 cm) at a flow rate of 1 ml per min. The mobile phase was a gradient of 15–45% solvent A for 30 min, followed by an increase to 88% solvent A, where solvent A is methanol, and solvent B is 0.1% acetic acid. Fractions were collected for biologic assay as well as structural identification of constituents by gas chromatography–mass spectroscopy and ^1H nuclear magnetic resonance.

Clinical evaluation in pest control workers Terminex pest control workers were invited to participate in a placebo-controlled trial. Either a balm containing SdG (1%, Zangrado Bug Bite Balm, Rainforest Phytoceuticals, LLC; http://www.amazonmedicines.com) or placebo (balm base without the SdG) was applied to various skin conditions encountered over a 3 mo period at the discretion of the participants. The balms were coded and their nature was not disclosed to the participants during the study. Because of their occupation, these pest control workers incur various cutaneous afflictions. Participants were also asked to keep a log of the type of afflictions, associated symptoms, time to achieve relief, complications or side-effects, and whether reapplication was necessary. A total of 11 participants were enrolled but only 10 participated in the study. The time frame was open-ended, but was concluded after 3 mo because the spring infestations had yielded a reasonable number of ''events''. Because of the random nature of these afflictions in a ''real world'' situation, participants were asked to apply either balm at their discretion. If for example, more than one bite occurred at any one time then participants were asked to apply both balms on different locations in order to facilitate comparison. Some events by their nature were isolated, however, and so balms were applied in series as opposed to in parallel. At the conclusion of the trial logged information was decoded and analyzed. No workers were encouraged to be attacked by insects, rather all ''events'' occurred as a hazard of their occupation.

RESULTS

CGRP vasorelaxation Rat mesenteric arteries, preconstricted with phenylephrine, relaxed in response to addition of CGRP to the superfusate ($pD2 = 8.84 \pm 0.08$, $n = 5$). Addition of SdG to the superfusate at a dilution of 1:10 000 resulted in a significant shift of the dose–response curve to the right ($pD2 = 8.08 \pm 0.1$; $n = 5$, $p < 0.01$), as indicated in **Fig 1**. Vasorelaxant responses to acetylcholine were unaltered by SdG (data not shown). Repetitive dose–response curves to CGRP in the absence of SdG were identical.

Rat paw edema Edema induced by intradermal injection of PAR_2-AP is the result of sensory afferent nerve activation, as this response can be abolished by neonatal capsaicin treatment (sensory afferent ablation), and neurokinin receptor antagonism (Vergnolle *et al*, 1999; Steinhoff *et al*, 2000). To address the potential effects of SdG, we applied a balm containing 1% SdG to the rat paw 20 min prior to PAR_2-AP injection, with edema monitored for a subsequent 6 h (**Fig 2**, $n = 8$ in each group). In both groups PAR_2-AP caused a marked increase in paw volume by 1 h, indicative of edema development. Paw volume then slowly

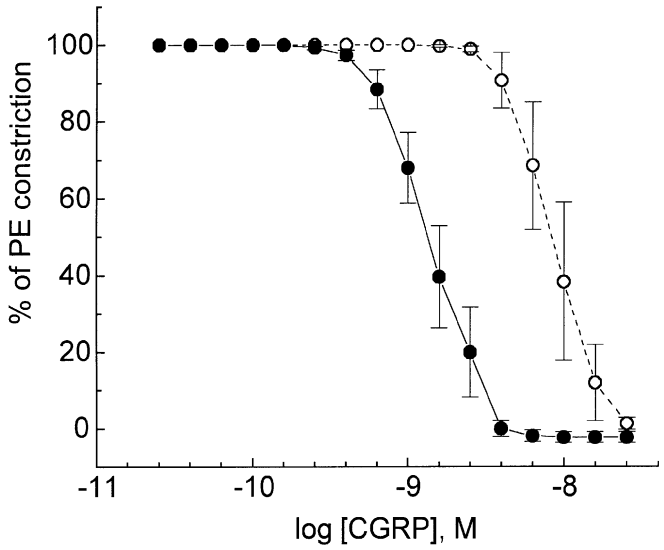

Figure 1. SdG and CGRP-induced vascular relaxation. Dose–response curves to CGRP in rat mesenteric arteries preconstricted with phenylephrine (PE). Data are expressed as a percentage of the PE tone. Vascular preparations were made from five animals in each group. Sequential dose–response curves to CGRP were superimposable (*closed circles*, data not shown). After establishing the response to CGRP alone SdG (1:10,000 dilution) was introduced into the superfusate, and the response to CGRP repeated. SdG caused a significant shift of the dose–response curve to the right (*open circles*, *broken lines*, $p < 0.05$). Results are depicted as mean ± SEM for five animals in each group.

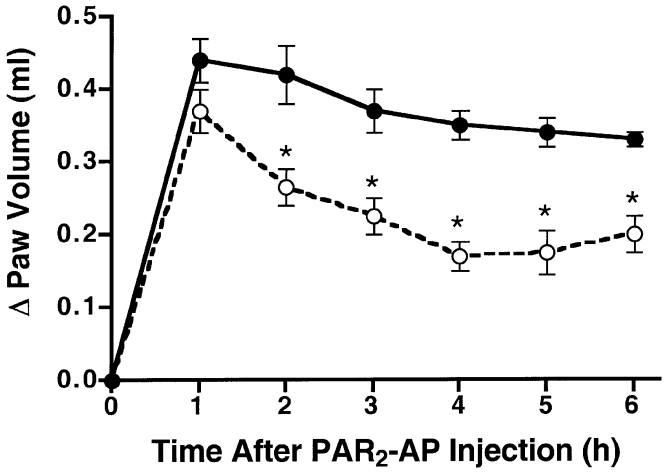

Figure 2. SdG and PAR-$_2$AP induced Edema Formation. PAR-$_2$AP used was SLIGRL-NH2. It was injected into the rat paw at a dose of 500 μg. Either the placebo balm (*closed circles*, *solid lines*) or the Zangrado Bug Bite Balm (*open circles*, *broken lines*) were applied topically (40 mg) to the paw 20 min prior to injection of the SLIGRL-NH_2. Paw volume was measured using a hydroplethysmometer, by an individual unaware of the treatments the rats had received. Results depict the mean ± SEM for eight rats in each group. The asterisk indicates a significant difference ($p < 0.05$) between placebo and Zangrado balm groups as determined by ANOVA and *post hoc* analysis with Student–Newman–Keuls t test.

declined over the next 5 h in the placebo group. In SdG-treated paws, the decline in paw volume was more dramatic and sustained despite its single topical application, resulting in approximately 50% less edema ($p < 0.01$, **Fig 2**).

Rat paw hyperalgesia Intradermal injection of PAR$_2$-AP resulted in a decrease in the latency withdrawal period to a heat

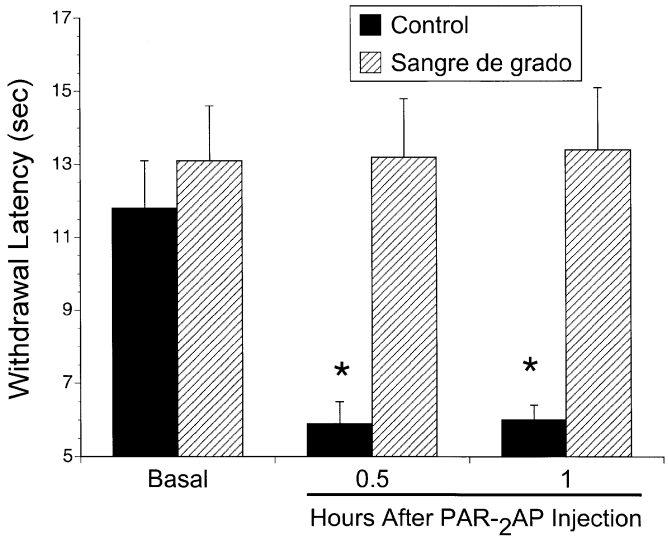

Figure 3. Hyperalgesia induced by PAR-$_2$AP and the effects of SdG. Intradermal injection of PAR-$_2$AP (50 μg) resulted in a state of hyperalgesia as indicated by a reduction in latency withdrawal time from baseline in rats treated with placebo balm (* $p < 0.05$ compared with basal values, n = 5, ANOVA and *post hoc* analysis with Dunnett's multiple comparison test). Zangrado balm (containing 1% SdG, n = 7) completely prevented the induction of hyperalgesia, with withdrawal latency being unaltered from baseline. Results are depicted as mean ± SEM.

source (**Fig 3**). This is indicative of a hyperalgesic state. Pretreatment with topical SdG (1%, Zangrado Bug Bite Balm) prevented the induction of hyperalgesia, with the latency withdrawal time remaining at its baseline level despite PAR$_2$-AP administration (**Fig 3**). Zangrado balm did not affect withdrawal latency time in rats that did not receive PAR$_2$-AP (data not shown) indicating that it was not acting as an anesthetic. Hyperalgesia was also induced by intradermal PGE_2, which is thought to induce an increased sensitivity to pain perception by raising the resting potential of sensory afferent nerve fibers. In these experiments intradermal PGE_2 resulted in a significant reduction in paw withdrawal time (**Fig 4**) and this effect was blocked by a single topical administration of SdG balm.

Potential thermal analgesic bioactivity of fractionated SdG was assessed using PAR$_2$-AP as the algesic agent. Two fractions of SdG were ineffective (fractions 2 and 3), with paw withdrawal times being indistinguishable from vehicle-treated animals (**Fig 5**). In contrast, fraction 5 was fully effective in preventing the hyperalgesic response to intradermal PAR$_2$-AP, comparable with that observed with the parent botanical SdG (**Fig 3**).

Capsaicin-induced hyperemia Gastric hyperemia in response to luminal capsaicin (320 μm) was evident in control animals, with an average increase in basal blood flow of 60% (**Fig 6**). In rats treated with either SdG or its derivative, fraction 5, this capsaicin-induced hyperemia response was largely abolished ($p < 0.01$). The concentration chosen for assessment, 1%, was based on the concentration that was effective topically in rat edema and analgesic assays as well as the clinical evaluation in pest control workers.

Structural determination of active chemical constituents Analysis of fraction 5, a fraction that shared the same biologic properties of the parent botanical, indicated that it was a pure compound with a molecular weight of 930, and likely to be an oligomer of proanthocyandins. Proanthocyandins are the predominant chemical component of SdG but yet, to date, proanthocyandins have not been reported to possess sensory afferent nerve suppressant activity. It should be noted that fraction 3, which was ineffective as an analgesic agent (**Fig 5**), possessed the same molecular weight and proanthocyandin

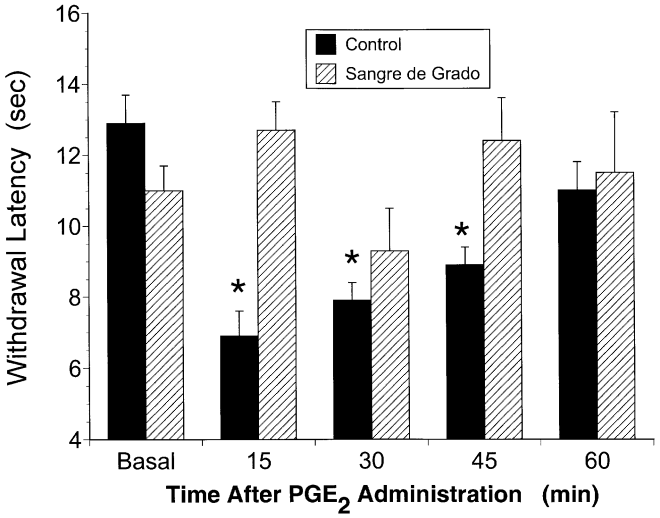

Figure 4. Hyperalgesia induced by PGE_2 and the effects of SdG. Intradermal injection of PGE_2 (0.3 μg) resulted in a state of hyperalgesia as indicated by a reduction in latency withdrawal time from baseline in rats treated with placebo balm (*$p < 0.05$ compared with basal values, n = 5, ANOVA and *post hoc* analysis with Dunnett's multiple comparison test). Zangrado balm (containing 1% SdG, n = 5) completely prevented the induction of hyperalgesia, with withdrawal latency being unaltered from baseline. Results are depicted as mean ± SEM.

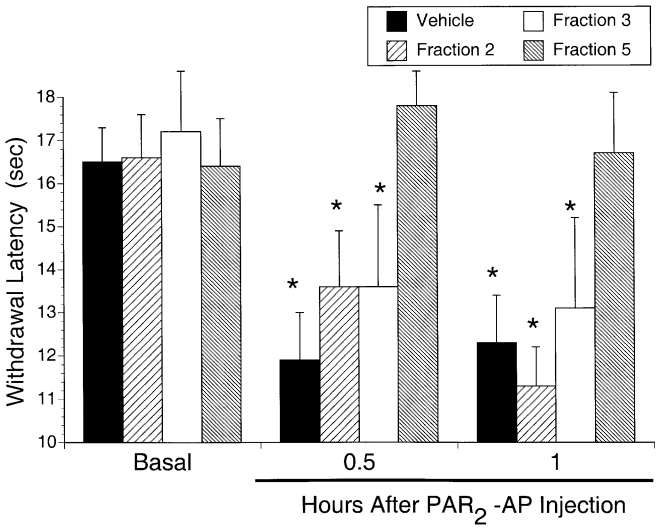

Figure 5. Effects of SdG fractions on the hyperalgesia induced by PAR_2-AP. Intradermal injection of PAR_2-AP (50 μg) resulted in the induction of thermal hyperalgesia as indicated by a reduction in latency withdrawal time from baseline in rats treated topically with the vehicle balm. Balm containing 1% of fractions 2, 3, or 5 isolated from the parent botanical, SdG, were evaluated at 30 and 60 min after PAR_2-AP administration. Fraction 5 completely prevented the induction of hyperalgesia, with withdrawal latency being indistinguishable from baseline. In contrast, fractions 2 and 3 were ineffective analgesic agents, producing similar results to the vehicle control. Results are depicted as mean ± SEM, n = 5 (*$p < 0.05$ compared with basal values, n = 5, ANOVA and *post hoc* analysis with Dunnett's multiple comparison test).

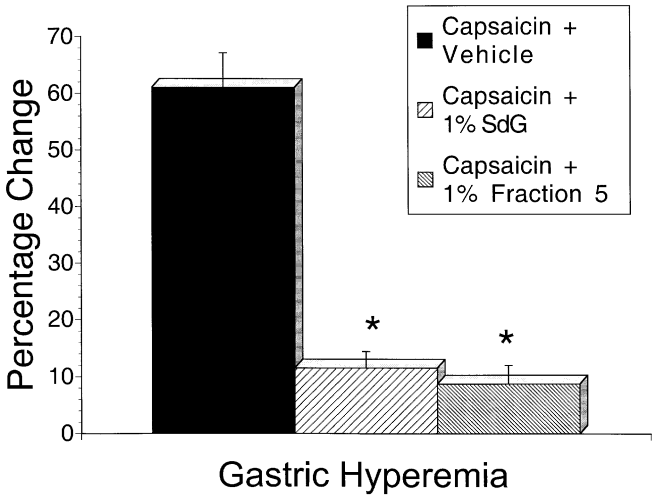

Figure 6. Effects of SdG and fraction 5 on the gastric hyperemia induced by luminal capsaicin. Gastric mucosal blood flow was measured by a laser Doppler flow meter in rats. Luminal exposure to capsaicin (320 μm) greatly increased mucosal blood flow (expressed as a percentage change from baseline, mean ± SEM). in rats pretreated with luminal SdG or fraction 5 (both 1% solutions), the hyperemic response to capsaicin was largely absent (n = 5, *$p < 0.01$ *vs* vehicle).

Table I. Afflictions experienced by pest control workers[a]

Affliction	Individuals reporting a specific affliction	Total no. of individuals enrolled
Bee	1	10
Wasps	6	10
Mosquito	1	10
Fire ant	10	10
Other ants	6	10
Plant reactions	1	10
Cut	5	10
Abrasion	1	10

[a]The number of individuals reporting an individual affliction is noted. Not all participants encountered the same profile of afflictions, as to be expected, as these events were random and reflect the occupational hazard. It is important to note that an individual participant may have experienced the same type of affliction multiple times, but it is only noted here once. This table only describes the profile of events that were recorded over a 3 mo period, and not the number of events that any single participant encountered.

oligomer base as fraction 5. In other words, it appears that only certain isomers of these proanthocyandin oligomers possess the desired activity, with some proanthocyandin isomers (fraction 3) having no analgesic activity, whereas others (fraction 5) display both analgesic and capsaicin antagonistic properties. At this time further comparative analysis of how the spatial arrangement of these complex oligomers is not available. Given the complex nature of the components of this botanical it should be noted that chemical constituents other than proanthocyandins may be active.

Clinical evaluation of a SdG-based balm An evaluation of a commercially available SdG balm (Zangrado Bug Bite Balm) for relief of the symptoms of insect bites, stings, and other skin conditions was done in Terminex pest control workers in New Orleans, LA. These workers are prone to these hazards as part of their occupation. Participants compared the effects of a placebo balm with a balm containing 1% SdG over a 3 mo period. As noted in **Table I**, the primary affliction during this period was fire ant bites, with all participants suffering at least one attack. Fire ants are endemic throughout the south-eastern United States, and they elicit an initial painful bite followed by an itching response that can persist for weeks. Because of the uncontrolled environment in which this study was performed every participant did not experience the same number or type of encounters. Some individuals encountered the same affliction multiple times (for example fire ants).

Table II. Profile of symptoms experienced by pest control workers and effects of placebo *vs* SdG (Zangrado) balm[a]

Symptoms	Individuals reporting	Relieved by placebo	Relieved by Zangrado	Preference (%)
Itching	10/10	0	10***	Zangrado (100)
Pain	5/10	1	5*	Zangrado (100)
Redness	6/10	1	6*	Zangrado (100)
Swelling	6/10	0	6**	Zangrado (100)
Discomfort	4/10	0	4*	Zangrado (100)

[a]The profile of symptoms was dependent upon the type of affliction that these workers encountered. For example, all participants were bitten by fire ants during the study and the primary symptom associated with fire ant bites is intense itching. Comparisons between placebo and Zangrado balms were performed using the Fishers Exact test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). All participants preferred the Zangrado balm over the placebo balm for symptomatic relief; on average relief was reported to occur in < 2 min.

The symptoms associated with these encounters are recorded in **Table II**. Itching (10 of 10 participants) was the predominant symptom, reflecting the preponderance of fire ant bites among the workers. The placebo balm offered relief in only two cases, whereas the SdG relieved symptoms in all individuals, for all applications. The SdG balm was preferred over placebo balm by all participants and relief was reported to occur in less than 2 min, on average. The diversity of conditions in which the SdG offered symptomatic relief was broad (**Table I**). That combined with the speed by which it achieved relief, is highly suggestive that benefit was related to a sensory afferent-based mechanism of action.

DISCUSSION

Despite the widespread use of SdG in Amazonia as an analgesic, antidiarrheal, and wound-healing agent few in the Western world are aware of its existence and little is known about how it achieves these therapeutic benefits. We postulated that these benefits may result from a suppression of sensory afferent nerve activation and the present results support this conclusion. This hypothesis was generated from experience and the knowledge that sensory afferent nerves serve as broad-based sentinels in the skin, gut, and lung, and that the rapidity by which SdG relieved pain and itching was consistent with a neurogenic mechanism. In addition, the serendipitous personal observation by an author that SdG relieved the symptoms of cutaneous capsaicin (sensations associated with an overly spicy meal) focused our attention to sensory afferent nerve mechanisms.

SdG appears to suppress the activation of sensory afferent nerves at a prejunctional level, in addition to inhibiting the tissue responses to CGRP, a primary neurotransmitter of sensory afferent nerves. Supporting the present findings we recently noted that SdG was able to attenuate the epithelial secretory response to capsaicin but not the neurokinin-1 antagonist, Sar-Met-substance P in Ussing chambers of guinea pig ileum (Miller *et al*, 2000). The use of guinea pig ileum in this study offered significant advantages for evaluating sensory afferent nerve mechanisms. Specifically, capsaicin-induced epithelial secretion is due solely to the release of substance P and subsequent activation of NK-1 receptors (Vanner and MacNaughton, 1995). Thus, as SdG did not alter NK-1 secretory responses but attenuated capsaicin-induced secretion, we can conclude SdG was operating at a prejunctional level, directly blocking the activation of sensory afferent nerves (Miller *et al*, 2000). This ability of SdG to block capsaicin epithelial secretory responses was extended in our study to include capsaicin-induced gastric mucosal vasodilation (**Fig 6**).

The conclusion that SdG is an inhibitor of sensory afferent nerve mechanisms is further supported by our studies using PAR_2-AP or PGE_2 as the hyperalgesic agents. A dose of PAR_2-AP (50 μg per kg) that does not elicit edema was used to avoid the complications of interpretation surrounding the potential anti-inflammatory actions of SdG influencing pain perception. Topical SdG in this setting completely prevented the hyperalgesia induced by PAR_2-AP (Vergnolle, 2000). PAR_2-AP has only recently been described as a means of direct activation of sensory afferent nerves (Steinhoff *et al*, 2000) and its precise mechanism is less well characterized than capsaicin.

Hyperalgesia induced by PGE_2 was also blocked by topical SdG. In contrast to PAR_2-AP, PGE_2 elicits hyperalgesia by indirect means, specifically PGE_2 sensitizes sensory afferent nerves to activation by other stimuli, but yet does not directly activate these nerves. From this collage of results we conclude that SdG is acting in a broad manner to suppress sensory afferent nerve activation. Whereas it is possible that SdG is acting as an antagonist for PAR_2-AP, this cannot explain its ability to block PGE_2 or capsaicin responses. The capsaicin or vanilloid receptor is thought to be a critical pathway for thermal hyperalgesia to various stimuli, as indicated by the absence of thermal hyperalgesia in mice with vanilloid receptor 1 (VR1) gene deletions (Caterina *et al*, 2000; Davis *et al*, 2000). Sterner and Szallasi (1999) noted that current medicinal plants invariably possess residual VR1 agonistic activity, termed pungency. This is not a problem noted with SdG as it did not raise basal gastric blood flow nor is it pungent to the taste. Whereas one explanation for the present observations is that SdG acts as a pure VR 1 antagonist with no agonist properties, there is no direct support for that conclusion, and alternative indirect pathways leading to sensory afferent nerve suppression must be considered.

It is interesting to note that SdG was an effective analgesic and anti-inflammatory agent when applied topically, even when the hyperalgesic stimuli were applied by intradermal injection. This suggests that active components have sufficient lipophilicity to readily cross the skin. From the observations from the pest control workers who noted symptomatic relief in less than 2 min, this transcutaneous absorption appears to be rapid. Fractionation studies indicated that fraction 5, which was an effective inhibitor of capsaicin-induced hyperemia, was an analgesic agent when applied topically. Whereas capsaicin-induced tissue responses may not always be neuronal in origin (Wallengren and Håkanson, 1987; Wallengren and Håkanson, 1992), the pattern and rapidity of SdG effects suggest that a neuronal action is likely.

Whereas it is generally recognized that medicinal plants exert their effects through a combination of chemical constituents we have attempted to isolate the active components in this natural product. Antioxidant oligomers of proanthocyandins have been reported to mediate the ability of SdG to attenuate epithelial secretion in response to increased epithelial levels of cyclic adenosine monophosphate and also as an inhibitor of viral replication (Ubillas *et al*, 1994; Gabriel *et al*, 1999). Proanthocyandins constitute the major chemical class in SdG, accounting for 90% of its composition. Proanthocyandins, however, are common components of numerous plants, e.g., they are responsible for giving red wine its characteristic color, a property shared with SdG. Wine-derived proanthocyandins have not been reported to block sensory afferent nerve activation, suggesting that either the proanthocyandins in SdG differ from other sources or other components are more important. A preliminary structural evaluation of active principles indicates that certain proanthocyandin oligomers may underlie the desired effects. One fraction, tentatively identified as a proanthocyandin oligomer with a molecular weight of 930, was analgesic and attenuated capsaicin responses *in vivo*; however, related oligomers with an identical molecular weight were devoid of analgesic activities. This paradoxical result suggests that the manner in which these complex oligomers are spatially arranged is critical for their biologic activity. The nature of this arrangement is yet to be determined. One must also consider that bioactivity lies in alternative chemical classes (Pieters *et al*, 1993).

The dual prejunctional and postjunctional effects of SdG on sensory afferent mechanisms (nerve activation and CGRP receptor

inhibition) are unique and are consistent with both the rapidity and breadth of SdG's benefits. Combined with its reported antimicrobial (Rao *et al*, 1982; Chen *et al*, 1994; Phillipson, 1995) and antiviral properties (Ubillas *et al*, 1994) it is timely to note that it is unlikely that no single chemical would share all these functions. SdG may be an example of how a natural product through its chemical diversity, may offer therapeutic advantages over a pharmaceutical. In the specific case of managing neurogenic inflammation the current approach is to treat with other phytochemical vanilloids, such as capsaicin or resiniferatoxin. The goal of this approach is to deplete the sensory afferent nerve terminals of neurotransmitters in order to limit their contribution to both the pain signal and the local tissue responses. This result is achieved, however, by the acute activation of the precise mechanisms that one is trying to block, and is in essence a clumsy approach to therapy. A more specific approach, however, has not been available. SdG offers a different approach to managing disorders characterized by excessive or sustained activation of sensory afferent nerves (neurogenic inflammation) – rapid suppression of nerve activation. Conditions in which SdG may offer new therapeutic options include eczema, psoriasis, contact dermatitis and hypersensitivity reactions and ultraviolet damage (Goebeler *et al*, 1994; Ansel *et al*, 1996; Wallengren, 1997; Quinlan *et al*, 1998; Scholzen *et al*, 1999). Potentially any skin condition characterized by itching, pain, edema, redness, and discomfort may receive benefit from an agent that inhibits sensory nerve afferent activity.

Ethnomedical applications for SdG are not confined to the skin (Maxwell, 1990). The oral intake of SdG for diarrhea and intestinal distress may well be due to its ability to modify sensory afferent mechanisms in the gut (Miller *et al*, 2000). Sensory afferent nerves are major components of the enteric nervous system, and contribute not only to secretion, but also to cramping, discomfort, and pain perception. One may speculate that SdG could also provide therapeutic benefit for various pulmonary disorders that involve sensory afferent nerves. These include airway hyperresponsiveness – cough, asthma (Reidel *et al*, 1997; Choi and Kwon, 1998; Tohda *et al*, 1998), and viral infections (Piedimonte *et al*, 1990, 1997; Yamawaki *et al*, 1995), provided an adequate delivery system could be designed. In addition, sensory afferent nerves and their neuropeptides have also implicated in the pathogenesis of arthritis (McDougall *et al*, 1999; Vilensky and Cook, 1998)

We conclude that SdG offers a valuable tool for determining the role of sensory afferent nerves in inflammation. Considering the recent advances provided by studies of the VR1 knock-out mice, demonstrating significant thermal analgesia resulting from the failure to activate sensory afferent nerves (Caterina *et al*, 2000; Davis *et al*, 2000), any botanical that suppresses sensory nerve activity warrants a detailed evaluation of its therapeutic potential. Indeed, SdG's actions as an analgesic and anti-inflammatory agent may provide a new and valuable therapeutic approach to a variety of inflammatory disorders and serves to highlight the valuable medicinal resources that are still contained within the rain forests of Amazonia.

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