December 2013, Vol. 7, No. 12, pp. 1243-1248
Journal of Life Sciences, ISSN 1934-7391, USA

In Vivo Anti-Inflammatory Assessment of *Quillaja saponaria* Mol. Extracts

Maité Rodríguez-Díaz[1], Olosmira Correa[2], Carlos Cartagena[3], Sylvia Arrau[3], Bruce K. Cassels[4] and Carla Delporte[3]

1. Faculty of Medicine, Universidad Andres Bello, Santiago 8370134, Chile

2. Department of Pharmaceutical Technology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago 1058, Chile

3. Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago 1058, Chile

4. Department of Chemistry, Faculty of Sciences, University of Chile, Santiago 1058, Chile

Received: June 03, 2013 / Accepted: August 12, 2013 / Published: December 30, 2013.

Abstract: Aqueous extracts from the native Chilean *Quillaja saponaria* tree are used orally and topically to treat lung inflammatory problems and toothache. This study aimed to corroborate their presumed anti-inflammatory activity, identify their active ingredients and validate their use. The topical anti-inflammatory activity of a commercial partially purified *Quillaja saponin* aqueous extract (Ultra Dry 100Q, UD), the crude acid hydrolysate (H-100Q) of this material, its two most abundant sapogenins QA (quillaic acid) and PA (phytolaccagenic acid), and two formulations containing QA were subjected to topical assays for the inhibition of murine ear inflammation elicited by AA (arachidonic acid) or TPA (phorbol ester). The dose-dependent anti-inflammatory activity of QA was confirmed in both AA (maximal effect 92.1%) and TPA (maximal effect 62.2%) assays, and PA showed significant anti-inflammatory activity against AA (46.5%). Two dermo pharmaceutical formulations containing 8% w/v QA as the active ingredient—a cream and a gel—also exhibited significant anti-inflammatory effects in the TPA (50.8%) and AA (39.5%) assays.

Key words: *Quillaja saponaria*, triterpenoids, quillaic acid, phytolaccagenic acid, topical anti-inflammatory activity, phorbol acetate, arachidonic acid.

1. Introduction

The bark of *Quillaja saponaria* Mol., Quillajaceae ("soap bark", "Seifenrinde", "Panama bark", "Bois de Panama") has been used from times immemorial by the Mapuche people, the major ethnic group of south-central Chile, to wash hair and wool [1] and for the treatment of toothache and respiratory inflammations [2]. Recently, this species has been intensively studied for its triterpene saponin content (between 8.5% and 16.4%). The more or less pure saponins and specific fractions of the same are widely used as vaccine adjuvants [3].

Saponins and sapogenins have been reported as bioactive ingredients in cosmetic formulations that supposedly delay the skin aging process [3, 4]. The anti-inflammatory activity of several pentacyclic triterpenoids, such as oleanolic acid, has been reported, supporting their use in traditional medicine [5-9]. 3β, 16α-Dihydroxy-23-oxoolean-12-en-28-oic acid (QA, quillaic acid) has been recognized, for at least eighty years, as the major aglycone of quillaja saponin followed in quantity by PA (phytolaccagenic acid). Recent studies have shown that all the *Quillaja* sapogenins possess the oleanane skeleton [10]. The topical anti-inflammatory activity of QA has been reported recently [11], and so have both the topical

Corresponding author: Carla Delporte, Ph.D., research field: chemical and pharmaceutical sciences. E-mail: cdelpor@uchile.cl.

and the intraperitoneal analgesic activity of QA [12].

In spite of the traditional use of saponin-rich aqueous extracts of this species to treat inflammation, no scientific studies are available, and the potential use of its major sapogenin as a dermopharmaceutical anti-inflammatory ingredient has not been explored thus far. The main aim of this study was to determine the topical anti-inflammatory activity of a commercial partially purified *Q. saponaria* saponin extract (Ultra Dry 100Q, UD) and its crude acid hydrolysate (H-100Q). In addition, the anti-inflammatory effect of PA and the dose-effect relationship of QA were examined, and two topical formulations of QA, a gel and a cream, were assayed.

The topical anti-inflammatory activity was evaluated in the mouse ear assay vs. AA (arachidonic acid)-induced and TPA (12-O-tetradecanoylphorbol-13-acetate)-induced inflammation. TPA acts primarily as an activator of protein kinase C and NF-κβ, promoting the enhanced expression of pro-inflammatory enzymes. On the other hand, AA presumably acts as a precursor of inflammatory mediators such as prostaglandins and leukotrienes [13-15].

2. Materials and Methods

2.1 General Procedures

Synthesis grade reagents and solvents were purchased from Merck (Darmstadt, Germany). UD (Ultra Dry 100Q) was donated by Natural Response S.A. (Quilpué, Chile). CC (column chromatography) was carried out using Merck silica gel 60 (0.015-0.040 mm), and analytical TLC (thin layer chromatography) was performed on Merck silica gel 60 F254 aluminium foils. Spots were detected by spraying with p-anisaldehyde/sulfuric acid reagent, followed by heating for 1 min at 120 ºC. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 126 MHz for QA, and ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, for PA, on commercial spectrometers. Chemical shifts (δ) are reported in ppm relative to the TMS signal, using the solvent as internal reference. MS (mass spectra) were obtained using a commercial instrument with an ESIMS (electrospray ionization source). For the pharmacological studies, two pro-inflammatory agents were used, TPA (12-O-tetradecanoylphorbol-13-acetate) and AA (arachidonic acid), both purchased from Sigma (St. Louis, MO). Reference drugs, indomethacin and nimesulide, were donated by Laboratorio Chile (Santiago, Chile).

2.2 Preparation of the Aqueous Extract and Sapogenins of Quillaja saponaria Mol.

To hydrolyze the aqueous extract, UD (20 g) was refluxed with 9% HCl (500 mL) for 3 h, cooled to room temperature and filtered. The solid (H-100Q) was washed three times with water and then dried to obtain a residue weighing 8 g. To obtain sapogenins QA and PA, H-100Q was subjected to CC, first on silica gel and then on Sephadex LH-20. The major fraction eluted from Sephadex was identified as QA, and a slightly more polar fraction was shown to be PA by comparison with standards [11], and on the basis of NMR and MS analysis whose results were in agreement with published data [16, 17].

2.3 Preparation of Formulations

Two formulations—a cream (O/W) and a gel—both containing 8% of QA, were prepared as follows:

(1) Cream formulation. All the aqueous phase material and the oil phase ingredients were placed in separate stainless steel containers and heated at 70 °C. The water phase was then added to the oil phase with continuous stirring. The semisolid emulsions (O/W) were then cooled to approximately 40 °C, and QA, previously dissolved in phosphate buffer (pH 6.8), was added together with other additives;

(2) Gel formulation. HEC (Hydroxyethyl cellulose, 4%) powder was added to distilled water at 70 °C with stirring, and the dispersion was allowed to cool. The QA previously dissolved in phosphate buffer (pH 6.8),

was dissolved in 5 g of 2-propanol and 5 g propylene glycol and mixed with the HEC dispersion with continuous stirring at 37 °C until the gel formed (2 h). Then it was completed with distilled water until 100 g.

2.4 Topical Anti-Inflammatory Activity

Adult male CF-1 mice (20-25 g) from a stock maintained at the Chilean Public Health Institute were used to assess the anti-inflammatory effect of the samples under study. All animals were kept on a 12 h light-dark cycle, with water and food provided *ad libitum* and fasting overnight before the experiments. All the animal experiments were performed according to the ethical guidelines suggested by the "International Guiding Principles for Biomedical Investigation with Animals", promulgated by the CIOMS (1990) and the regulations of the Bioethics Committee of the Chilean Public Health Institute.

The topical anti-inflammatory activity was assessed *in vivo* as described by Delporte et al. [18]. Briefly, groups of 8 animals were treated with a single dose of each test compound dissolved in acetone (equimolar doses with regard to the reference drugs), topically applied on the inner (10 L) and outer (10 L) surfaces of the right ear of the animals. After 5 min, 2 mg of AA or 5 μg of TPA, both dissolved in acetone, were topically administered on the right ear, using acetone on the left ear as a solvent control. In order to prevent alterations in the active ingredient absorption through the skin, the test compounds were applied before using either pro-inflammatory agent.

The control animals were treated similarly, but they did not receive the test compounds. Two other groups of 8 animals were treated with nimesulide or indomethacin dissolved in acetone, drugs used as references for topical inhibition of inflammatory activity induced by AA or TPA, respectively. After 1 h and 4.5 h for AA and TPA, respectively, all the animals were sacrificed by cervical dislocation and a section of 6 mm diameter of the right and left ears was punched out and weighed. The dermal anti-inflammatory effect (%EAI) was evaluated according to the following Eq. (1):

$$\%EAI = [W_c - W_t/W_c] \times 100 \quad (1)$$

where W_c and W_t are the medians of the weight differences of the right and the left ear sections for control and treated animals, respectively. Least-squares linear regression analysis of the log-dose response curves allowed the calculation of the dose or concentration that produced 50% of anti-inflammation (ED_{50} for each QA). The doses evaluated for QA were 0.7 mg/ear against TPA and 1.6 mg/ear against AA.

2.5 Statistical Analysis

Data were expressed as median values ± SEM calculated from the weight of the ear disks for treated and untreated animals considering control values as 100% inflammation. Statistical significance of more than two groups was evaluated using the Kruskall-Wallis test, followed by Dunnett's multiple test for individual comparisons (free PRISM software was used). The criterion for statistical significance was set at $P \leq 0.05$ [19].

3. Results and Discussion

H-100Q was obtained as a dark brown solid corresponding to about 30% of the UD. The yield of QA was about 17.5% of the H-100Q. The pharmacological results for UD, H-100Q, PA, QA and its formulations are shown in Table 1. While UD did not exhibit topical anti-inflammatory activity in either assay, H-100Q was significantly active against TPA and less so against AA. QA had already been shown to exhibit strong anti-inflammatory effects in both models [11] (Table 1). Our present results show that PA is also active, although not as potent as QA (Table 1). The cream and gel containing 8% QA were administered at the single dose of 20 L/ear, i.e., 1.6 mg/ear. Both proved effective, but so less than the QA acetone solutions.

The topical anti-inflammatory effect of QA was

Table 1 Results of topical anti-inflammatory effect against AA and TPA of UD, H-100Q, PA, QA and its formulations.

Sample	Doses	$\%EAI_{AA} \pm SEM$	$\%EAI_{TPA} \pm SEM$
Nimesulide (AA)	1.0 mg/ear	↑ 48.8* ± 4.0	5.0 ± 5.6
Indomethacin (TPA)	0.5 mg/ear	0.0 ± 9.0	↑ 92.9* ± 3.2
UD	1.5 mg/ear	19.8 ± 13.3	0.0 ± 22.8
H-100Q	0.8 mg/ear	25.0 ± 8.0	35.7* ± 5.5
PA	0.7 mg/ear	46.5* ± 10.0	27.3 ± 7.1
QA (AA) †	1.6 mg/ear	↑ 92.1* ± 4.2	-
QA (TPA) †	0.7 mg/ear	-	↑ 62.2* ± 16.6
QA—gel 8% w/v	20 μL/ear	32.4* ± 10.5	50.8* ± 11.2
QA—cream 8% w/v	20 μL/ear	42.6* ± 10.5	39.5* ± 10.4

Each value represents the median ± SEM of the results obtained from eight animals treated with samples or reference drugs. * $P \leq 0.005$, $n = 8$; ↑ maximal effect; EAI_{AA} = Anti-inflammatory effect against AA; EAI_{TPA} = Anti-inflammatory effect against TPA; † from Ref. [10].

Table 2 ED_{50} and log ED_{50} values for the antiinflammatory effect against AA and TPA, and maximal effect (%EAI) for topical administration of QA (quillaic acid).

Drug	ED_{50} (mg) ± SEM	Maximal effect %EAI ± SEM
Nimesulide (AA)	1.33 ± 0.02	48.8* ± 14.0
Indomethacin (TPA)	0.13 ± 0.03	92.9* ± 3.2
QA (AA)	0.60 ± 0.01	92.1* ± 4.2
QA (TPA)	1.0 ± 0.09	62.2* ± 16.6

ED_{50}: Dose that produced 50% of anti-inflammatory effect. * $P \leq 0.005$.

now assessed at different QA doses. The anti-inflammatory effect was dose-dependent, reaching a maximum effect of 92.1% against AA 1.6 mg/ear, and it was 62.2% against TPA at 0.68 mg/ear. Table 2 shows the ED_{50} calculated from dose-response curves for QA. The strongest effect of QA against AA (92.1%) greatly exceeded the maximum achieved by the reference drug nimesulide (48.8%). The strongest effect of QA against TPA was 62.2%, less than that of indometacin, but reached at a lower dose than that which proved maximally effective against AA.

In vivo pharmacological studies of QA using the topical route allowed us to obtain a preliminary estimate of the potential of this natural compound for more advanced work in animals. These studies are fundamental for later clinical studies. Additionally, the use of two inflammatory agents—TPA and AA—has yielded data that provide some insight into the levels at which QA interferes with the inflammatory cascade.

As to the reference drugs used, indomethacin is a potent COX inhibitor, while nimesulide is fairly weak in this regard. Nevertheless, indomethacin is much more potent in anti-inflammatory tests than expected from its ability to inhibit COXs, suggesting that it acts largely at an early stage in the cascade of events leading to inflammation. This is borne out by the fact that, in topical assays like those used by us, indomethacin is very active against TPA-induced inflammation but is practically inactive in the AA model. In contrast, nimesulide is effective in the latter model and shows little, if any, activity against inflammation elicited by TPA. The inflammatory process elicited by TPA develops much more slowly than AA-induced inflammation, presumably because TPA acts primarily as an activator of protein kinase C and NF-κβ, promoting the enhanced expression of proinflammatory enzymes such as the iNOS and COX-2. On the other hand, the action of AA is most probably exerted downstream as a precursor of inflammatory prostanoids [13-15].

Inhibition of AA- or, more usually, TPA-induced inflammation has been demonstrated over the last two

decades for a number of pentacyclic triterpene acids with the ursane, oleanane, and lupane skeletons, all of these from various medicinal plants. Some of them exhibited potent activity when compared with synthetic non-steroidal anti-inflammatory drugs [20]. Older reports had already shown that the anti-inflammatory activity of triterpenoids depends largely on the method used to generate inflammation, with a stronger effect against TPA-induced inflammation [15]. According to recent reports, some natural products diminish the release of early mediators of TPA-induced inflammation in mouse ear [21, 22].

Our studies lend support to previous results in the sense that most of our triterpenoids were more active in TPA- than in AA-induced inflammation, suggesting that they might be acting at an early stage, such as inhibition of NF-κB activation [11]. A particularly well studied example of a pentacyclic triterpenoid exerting its anti-inflammatory action by this mechanism is α-amyrin, which seems to act by suppressing COX-2 expression via inhibition of ERK, p38 MAPK and PKCα, and blockade of NF-κB activation [22]. However, QA also proved to be very effective in the AA-induced inflammation model. QA also shows analgesic activity [12], and we suggest that its mechanism of action might involve the inhibition of COX-2 and iNOS activity. In relation to the sapogenin (mostly QA)-rich H-100Q, the 0.75 mg/ear dose also exhibits a stronger effect against TPA- than against AA-induced inflammation. The low solubility of H-100Q did not allow the testing of higher doses.

The anti-inflammatory activity of the cream and gel containing QA is weaker than that of pure QA at similar concentrations. It seems likely that the simple excipients used reduce the bioavailability of the active compound, and that this drawback might be circumvented by the inclusion of absorption enhancers in the formulations. This loss of potency is more marked in the case of inflammation elicited by AA. This might be a consequence of the rapid inflammation induced by AA that could further delay the absorption of QA.

4. Conclusion

The current study confirms that QA is a highly effective inhibitor of *in vivo* inflammation induced by topical application of either TPA or AA and demonstrates that PA is also active, although less so. The maximal effect of QA against AA-induced inflammation is stronger than that of the reference drug nimesulide. The crude hydrolysate H-100Q, containing both QA and PA, presented weaker anti-inflammatory activity than the purified sapogenins against inflammation elicited by AA or TPA. QA can be formulated as an O/W emulsion (cream) or water-soluble gel in which its topical anti-inflammatory activity is preserved against both AA- and TPA-induced inflammation. The fact of presenting by both anti-inflammatory and analgesic effect shows the possible mechanism of action is through inhibition of PGE2 synthesis. Also we propose to investigate other formulations that allow the QA to maintain its effect.

Acknowledgments

Funding: This work was supported by FONDECYT Grant N1080174 and ICM Grant P05-001-F.

The authors acknowledge a generous gift of partially purified *Quillaja* saponin extract from Natural Response S.A. (Chile). The authors thank the Public Health Institute (Chile) for providing the experimental animals and Claudio Telha for his assistance in writing the paper.

References

[1] K. Kubitzki, The Families and Genera of Vascular Plants, Vol. 9, Springer, Berlin & Heidelberg, New York, 2007, pp. 407-408.

[2] J. Zin, C. Weiss, La Salud Por Medio de Las Plantas Medicinales, 6th ed., Editorial Salesiana, Santiago de Chile, 1980, p. 277. (in Spanish)

[3] M.R. Roner, J. Srayberry, M. Spinks, S. Dhanji, Antiviral activity obtained from aqueous extracts of Chilean

soapbark tree (*Quillaja saponaria* Mol.), Journal of General Virology 88 (2007) 275-285.

[4] S.S. Ajazuddin, Applications of novel drug delivery system for herbal formulations, Fitoterapia 81 (2010) 680-689.

[5] M. Aguirre, C. Delporte, N. Backhouse, S. Erazo, M. Letelier, B.K. Cassels, et al., Topical anti-inflammatory activity of 2α-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*, Bioorganic Medicinal Chemistry 14 (2006) 5673-5677.

[6] S. Böttger, M.F. Melzig, Triterpenoid saponins of the caryophyllaceae and illecebraceae family, Phytochemistry Letters 4 (2011) 59-68.

[7] J. Connolly, R. Hill, Triterpenoids, Natural Product Response 17 (2000) 463-482.

[8] R. Csuk, A. Barthel, K. Kluge, D. Ströhl, H. Kommera, R. Paschke, Synthesis and biological evaluation of antitumour-active betulin derivatives, Bioorganic Medicinal Chemistry 18 (2010) 1344-1355.

[9] M.F. Otuki, F. Vieira-Lima, F. Malheiros, R.A. Yunes, J.B. Calixto, Topical anti-inflammatory effects of the ether extract from *Protiumkleinii* and α-amyrin pentacyclic triterpene, European Journal of Pharmacology 505 (2005) 253-259.

[10] G.C. Kite, M.J. Howes, M.S. Simmonds, Metabolomic analysis of saponins in crude extracts of *Quillaja saponaria* by liquid chromatography/mass spectrometry for product authentication, Rapid Communication Mass Spectrometry 18 (2004) 2859-2870.

[11] M. Rodríguez-Díaz, C. Delporte, B.K. Cassels, P. González, X. Silva, F. León, et al., Topical anti-inflammatory activity of quillaic acid from *Quillaja saponaria* Mol. and some derivatives, Journal of Pharmacy and Pharmacology 63 (2011) 718-724.

[12] S. Arrau, C. Delporte, C. Cartagena, M. Rodríguez-Díaz, B.K. Cassels, P. González, et al., Antinociceptive activity of *Quillaja saponaria* Mol. saponin extract, quillaic acid and derivatives in mice, Journal of Ethnopharmacology 133 (2011) 164-167.

[13] J. Ban, J.H. Oh, T.M. Kim, D.J. Kim, H.S. Jeong, S.B. Han, et al., Anti-inflammatory and arthritic effects of thiacremonone, a novel sulfur compound isolated from garlic *via* inhibition of NF-κB, Arthritis Response Therapy 11 (2009) 145-146.

[14] A. Ray, M.V. Huisman, J.T. Tamsa, The role of inflammation on atherosclerosis, intermediate and clinical cardiovascular endpoints in type 2 diabetes mellitus, European Journal Intern. Medicine 20 (2009) 253-260.

[15] M.C. Recio, R.M. Giner, S. Mañez, J.L. Ríos, Structural requirements for the anti-inflammatory activity of natural triterpenoids, Planta Medica 61 (1995) 182-185.

[16] S.J. Guo, L. Kenne, Characterization of some *O*-acetylated saponins from *Quillaja saponaria* Molina, Phytochemistry 54 (2000) 615-623.

[17] N.T. Nyberg, L. Kenne, B. Rönnberg, G. Sundquist, Separation and structural analysis of some saponins from *Quillaja saponaria*, Carbohydrate Research 323 (2000) 87-97.

[18] C. Delporte, N. Backhouse, S. Erazo, R. Negrete, P. Vidal, X. Silva, et al., Analgesic-antiinflammatory properties of *Proustia pyrifolia*, Journal of Ethnopharmacology 99 (2005) 119-124.

[19] M. Hollander, D. Wolfe, Nonparametric Statistical Methods, J. Wiley & Sons, New York, 1973, pp. 27-32, 62-70.

[20] M. Ukiya, T. Akihisa, K. Yasukawa, K. Koike, A. Takahashi, T. Suzuki, et al., Triterpene glycosides from the flower petals of sunflower (*Helianthus annuus*) and their anti-inflammatory activity, Journal of Natural Product 70 (2007) 813-816.

[21] R. Medeiros, M. Otuki, M.C. Avellar, J. Calixto, Mechanisms underlying the inhibitory actions of the pentacyclic triterpene-amyrin in the mouse skin inflammation induced by phorbol ester 12-O-tetradecanoylphorbol-13-acetate, European Journal Pharmacology 559 (2007) 227-235.

[22] M.J. Wu, L. Wang, H.J. Ding, C.Y. Weng, J.H. Yen, *Glossogyne tenuifolia* acts to inhibit inflammatory mediator production in a macrophage cell line by down regulating LPS-induced NF-kB, Journal of Biomedicine Science 11 (2004) 186-199.