Anti-Inflammatory Actions of Pentacyclic Triterpenes

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Received: March 7, 1997; Revision accepted: July 10, 1997

Abstract: Pentacyclic triterpenes (PTs) as aglycones of saponins have a wide distribution in plants, and many of them have been used as anti-inflammatory remedies in folk medicine. This survey critically reviews the effects of PTs on proinflammatory mediator signalling pathways and data from experimental animal models and clinical trials. Because the knowledge of their actions is far from being satisfactory a critical summary of the partly promising but mostly scattered and preliminary data might promote productive research on chances and risks of PTs. Antiproliferative and anti-infectious actions and effects on intracellular cell signalling and hormone metabolism are beyond the scope of this short review, although such effects might also contribute to the understanding of the systemic anti-inflammatory actions of aglycones.

Key words: Pentacyclic triterpene pharmacology, terpenoids, sapogenins, inflammation, anti-inflammatory activity.

Introduction

In this review we present a survey on the antiphlogistic potential of a selected number of PTs (Fig. 1) for which the observed anti-inflammatory actions can be rationalized by data from in vitro mechanistic studies. We mainly consider studies which were carried out with purified compounds. We also try to reflect the reasonableness of the concentrations used in vitro and structure-activity relationships as far as data are available. Effects of saponins or crude extracts are only discussed if they contribute to the understanding of the actions of PTs.

Inflammation

The symptoms of an inflammation (redness, edema, heat, pain, and disturbed tissue function) are results of complex pathophysiological processes that include increased blood flow and vascular permeability, activation of humoral and cellular defence mechanisms, sensibilization and activation of nociceptors. These processes are mediated by a variety of signalling molecules produced by mast cells, macrophages, granulocytes, platelets, lymphocytes, nerve endings, endothelial cells, etc., as well as by the activation of complement factors. The mediators belong to different chemical classes, such as biologically active amines (histamine, serotonin), proteins and peptides (hydrolytic enzymes, cytokines, growth factors, colony stimulating factors, complement factors, antibodies, kinins), activated oxygen species (superoxide anion, hydroperoxide, hydroxyl radicals), and lipids (platelet activating factor, prostanoids, leukotrienes). These mediators initiate, maintain, aggravate, and modulate the course of a vast number of human disorders. Besides the huge number of proinflammatory players, the complexity of such processes is further increased by the involvement of endogenous antiphlogistic and immunomodulatory mechanisms (e.g., the release of anti-inflammatory and immunosuppressive steroids from the adrenal cortex) and by the fact that among one class of mediators some members might act anti-inflammatory: e.g., 15(R)-HETE and lipoxins from the products of the arachidonic acid cascade or the interleukins 4, 10, and 13 from the cytokine family, which exert pro- and contrainflammatory actions depending on their involvement in specific immune responses.
**Principles of anti-inflammatory drug action**

Anti-inflammatory compounds can act on many steps of pathophysiological processes. For example, a compound might block the biosynthesis of proinflammatory mediators by direct interaction with a key enzyme (e.g., inhibition of cyclooxygenase-II) or by decreasing enzyme expression (e.g., steroidal anti-inflammatory compounds) or by reducing substrate levels (e.g., decreased release of arachidonic acid). Alternatively, or in addition, a compound can act by inhibiting the release of preformed, stored mediators (e.g., histamine release) or block mediator receptor interaction on target cells (e.g., leukotriene receptor antagonists). An anti-inflammatory compound may also act by immunostimulation (e.g., maturation of myeloid cells, stimulated phagocytosis) that in turn promotes an increased removal of the insulting signal molecules (organisms or, alternatively, by immunosuppression) that results in a less aggressive inflammatory response to allergen challenge. Thus, to evaluate the potential and the therapeutic index of a compound, qualified information on the mechanism and the selectivity of its action is needed.

**PT Actions on Mediator Signalling**

**Histamine release**

Histamine is a preformed proinflammatory mediator stored in mast cells and basophilic granulocytes. Once released, it contracts smooth muscles of bronchi and gut, but powerfully relaxes those of small blood vessels. It promotes edema formation and stimulates gastric acid secretion and sensory nerve endings.

GA at concentrations from 20 to 35 μM, but not Gl, inhibited dexamethasone induced increase in histamine content and histidine decarboxylase activity in mastocytoma P815 cells, and decreased histamine release from antigen stimulated rat mast cells (1). UA at concentrations of 1000 μM or greater inhibited compound 48/80 induced histamine release from mast cells in vitro (2, 3). At 100 μM UA was only a weak inhibitor (26%), whereas other tested triterpenoids (i.e., betulinic acid, 3-O-acetylbetulinic acid, betulinaldehyde and ursolaldehyde) exerted no significant effects (2).

**Hydrolitic enzymes**

Under physiological conditions, endogenous and host macromolecules are intracellularly degraded by controlled, compartmented hydrolitic enzyme activities. Increased secretion of stored proteolytic enzymes from stimulated proinflammatory cells occurs in the course of pathophysiological processes. This sustained extracellular enzyme activity impairs functionally relevant macromolecules such as structure proteins, glycoproteins, and polysaccharides. Hydrolitic activities are also involved in complex regulatory processes like the release of biologically active compounds from precursor proteins, activation of complement factors, and degradation/activation of signal molecules, hormones, and receptors.
Table 1  Effects of pentacyclic triterpenes on hydrolytic enzymes: human leukocyte elastase (HLE); C3-convertase (C3C); hyaluronidase (HYU). IC₅₀ or Kₜ values are shown in µM and references in parentheses.

<table>
<thead>
<tr>
<th>enzyme</th>
<th>HLE</th>
<th>C3C</th>
<th>HYU</th>
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<tbody>
<tr>
<td>UA</td>
<td>2–4</td>
<td>&gt;200</td>
<td>300</td>
</tr>
<tr>
<td>OA</td>
<td>6</td>
<td>&gt;500*</td>
<td>280</td>
</tr>
<tr>
<td>AKBA</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uvaol</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrodiol</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hederagenin</td>
<td>62</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>18β-GA</td>
<td>185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escinol</td>
<td>1650</td>
<td></td>
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</table>

* A nonstandardized extract declared as boswellic acid was tested.

Human leukocyte elastase (HLE), which is produced and released by stimulated PMNL, is a highly aggressive and destructive serine protease. It has been suggested that HLE plays a role in several chronic disorders like pulmonary emphysema, cystic fibrosis, bronchitis, acute respiratory distress syndrome, glomerulonephritis, hepatitis, and rheumatic arthritis. HLE is believed to participate in the destruction of elastin, which is the primary elastic component of the lungs, blood vessels, and many other organs, and further proteins like fibronectin, laminin, collagen, proteoglycans, and immunoglobulins. The hydrolysis of blood vessel elastin by HLE promotes the transendothelial migration of stimulated proinflammatory cells. As shown in Table 1, UA, OA, BAs (especially AKBA), and uvaol (Fig. 2) in concentrations less than 20 µM inhibited HLE in vitro, whereas other PTs were less active (4, 5). The effect of PTs on HLE is the result of the interaction of these compounds with the extended substrate binding site of the enzyme. The type of inhibition is competitive or noncompetitive depending on both the substrate length and the substituents on the pentacyclic ring system (4, 5).

OA and hederagenin (with IC₅₀ values of 5 and 41 µM) inhibited the serine protease porcine pancreatic elastase (PPE) (6). UA inhibited PPE and trypsin with Kₜ values of 180 and 157 µM, respectively (4). The pancreatic serine protease chymotrypsin was weakly inhibited by UA in vitro (Kₜ and IC₅₀ values of 37 and 70 µM), but not by AKBA in concentrations up to 100 µM (4, 5). Application to rats of both the B. serrata gum resin and BA containing extracts thereof reduced glycosaminoglycan biosynthesis and degradation (7).

Strikingly, the gum resin was found to afford more therapeutic value than the BA extract in producing increased lysosomal stability and reduced β-glucuronidase activity (8). Effects on hyaluronidase of OA, hederagenin, and escinol were reported, but the IC₅₀ values for this effect were exorbitantly high (6). Effects of some PTs (e.g., OA ad BAs) on C3-convertase were observed but in these studies the inhibitors were used in concentrations greater than 200 µM (9, 10). A series of PTs inhibited immunohemolysis promoted by the classical complement pathway. Actions with IC₅₀ values in the range of 10 to 50 µM were reported for UA, β-BA, and crategolic acid, whereas GA and OA were less and β-amyrin and friedelin were not inhibitory (11).

In the alternative pathway high concentrations of β-BA (50 and 100 µM) exerted anticomplementary activity, whereas UA, OA, crategolic acid, and GA were less active (11). It was suggested
that the effects of PTs on complement activation might be due to
the inhibition of C3-convertase, which probably is the reflec-
tion of a more general inhibition of serine proteases (4, 11).

Cytokines

Cytokines (tumor necrosis factors, interleukins [ILs], in-
terferons [IFNs], colony stimulatory factors), which are released
by activated cells, have a wide range of effects that regulate
immune responses and inflammatory processes in a sustained
way. In target cells they modulate pathophysiologically re-
levant cell functions, such as adhesion molecule expression, cell
growth, division, differentiation or programmed cell death,
immunoglobulin production, chemotaxis.

From data of an early observation that the i.v. administration of
GL induced an antiviral activity in mice serum, it was deduced
that GL induced IFN, probably IFNγ production, GA promoted
antiviral activity was more moderate and not dose-dependent
(12). GL increased in concentrations of 10 to 100 μg/ml (about
12 to 120 μM) IFNγ production in concanavalin A stimulated
human peripheral lymphocyte-macrophage cocultures but not
in resting cells (13). In GL treated cells RNA and protein syn-
thesis were increased and the DNA synthesis was reduced. GL
(200 μg/ml, about 240 μM) prolonged the duration of the
growth response of T-lymphocytes, which were stimulated by
anti-CD3 monoclonal antibody, concanavalin A, or A23187 plus
PMA, along with increased IL-2 production and IL-2 receptor
expression. Since GL did not modulate receptor mediated pro-
tein tyrosine phosphorylation or c-fos transcripion, it was sug-
gested that the effect of GL is due to an interaction with a late
step of the signal transduction pathway for IL-2 production
(14). However, in immature thymocytes GL accelerated IL-2
production on the one hand and inhibited growth response on
the other hand. These actions in immature cells were
accompanied by an inhibition of tyrosine phosphorylation of
p40 and c-fos expression (15).

Reactive oxygen species and lipid peroxidation in vitro

In vivo, reactive oxygen species (ROS), such as NADPH-
oxidase-generated O2•− and enzymatic and non-enzymatic
products from O2•− are important factors of the cellular defence
mechanisms in phagocytes. Substrate conversion by mono- and
dioxygenases also contributes to the production of radicals and
peroxides. A sustained overboarding production of radicals and
peroxides may exhaust the capacity of endogenous scavengers
and anti-oxidative mechanisms and lead to an impairment of
functionally relevant structures. In in vitro and in vivo assay
systems radicals and peroxides are produced by chemical sys-
tems or by microsomal monooxygenase catalyzed metabolism of
drugs.

GL (60 nM to 6 μM) inhibited ROS generation in phagocytosing
human neutrophils in vitro, but did not exert ROS scavenger
activity in the xanthine/xanthine oxidase (XOD) system or impair
neutrophil phagocytosis and chemotaxis (16). High concentra-
tions (about 200 to 2000 μM) of 18β- and 18α-GA prevented
CCL4 and GalN induced cytoxicity in primary cultured rat he-
patocytes and Fe/ADP and Fe/ascorbate promoted lipid
peroxidation, but were ineffective at 20 μM. Strikingly, GA, in
contrast to vitamin E, did not suppress auto-oxidation of
linoleic acid. It was suggested that the action of GA was elicited
only in the case with lipids constituting the membrane struc-
ture (17).
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systems by a competitive mechanism (observations not published).

In vivo, carbenoxolone treatment was associated with elevated gastric luminal concentrations of PGE₂ in peptic ulcer patients (31). It was suggested that carbenoxolone inhibits the activities of 15-hydroxy-Δ₁₂-PAH-dehydrogenase and Δ₁₅-PG-reductase and that the resulting decreased inactivation of cytoprotective PGs synthesized within the gastric mucosa contributes to the postulated ulcer healing effect of carbenoxolone (32, 33). In addition, carbenoxolone inhibited the increased gastric mucosal release of LTC₄, which was induced by intragastric installation of ethanol (34).

Experimental Animal Models

Systemic anti-inflammatory effects of PTs might be due to their actions on mediator signalling discussed so far. However, distinct irritants given, interaction with steroid hormone metabolism, kinases, phosphatases, virus replication and tumor cell proliferation, though not discussed in this short review, might also contribute to anti-inflammatory actions. Examples of such effects are the inhibition of 11β-HSD by GA in the nanomolar range and with less efficiency by OA (35–37), the inhibition of protein kinases in the lower micromolar range by GA, UA, OA, and betulinic acid (38), and the inhibition of virus replication by GA and betulinic acid derivatives (Fig. 3) (39, 40).

Hepatoprotective activity

Experimental hepatitis in animals is induced by different mechanisms. Heavy metals and toxic metabolites of drugs, the latter are radicals and peroxides generated by microsomal enzymes, impair hepatocytes directly whereas the effects of galactosamine (GaIN) and endotoxin (LPS) are mediated by mechanisms including immunological responses.

Oral administration of 18β-GA, but not of GL, protected rats against GaIN induced increase in serum transaminases, whereas both GA and GL were active when administered i. p. (41). GL in rats (i. v. or i. p.) had no protective effect on hepatic injury induced by LPS but prevented the effects of CCl₄ and allyl formate (42).

A mixture of acetyl-BAs prevented GaIN and LPS induced liver damage in mice (43). Inhibition of HLE activity in addition to decreased formation of cysteinyl-LTs has been suggested to be the rationale for liver protection by BAs (5). OA protected rats against CCl₄-induced acute liver injury (44) and CCl₄-alcohol-induced cirrhosis (45) and CF-1 mice against hepatotoxic effects of CCl₄, acetaminophen, cadmium, bromobenzene, thioacetamide, furfurosemide, phalloidin, colchicine, GaIN, and LPS, but was ineffective against allyl alcohol (46, 47). Protection against acetaminophen was not evident until 24 h after s. c. injection of OA and lasted for 72 h (48). It was suggested that OA decreases the formation of toxic metabolites of acetaminophen and increases detoxication since in mice OA suppressed liver microsomal cytochrome P450 isoenzymes CYP1A and CYP2A and increased hepatic content of metallothionein (49, 50). Substantial protective effects against CCl₄-, acetaminophen-, and Cd-induced liver injury were also observed with α-hederin and Uva. Uvaol protected against CCl₄ and Cd-induced hepatotoxicity, GA, α-GA, and β-GA against acetaminophen, whereas hederagenin and 19α-hydroxyasiasic acid did not protect against any of the toxicants (51).

Acute inflammatory animal models

Anti-inflammatory effects in animal models were reported for a number of PTs. Most frequently the increase in paw volume of rats or mice in response to injection or implantation of irritants (carrageenan, dextran, cotton pellets, formaldehyde, serotonin, bacteria, etc.) has been measured. Since edema generation is most prominently promoted by proinflammatory PGs direct and indirect COX-II inhibitors (steroidal and nonsteroidal anti-inflammatory drugs) are often used as model compounds in these tests. It is noteworthy that many proinflammatory cytokines as well as many chemical and biological irritants upregulate COX-II expression.

GA and derivatives (30, 52), OA (53), betulin, betulinic acid, UA (54), and a crude BA containing extract from B. serrata gum resin (10, 55, 56) were reported to inhibit experimental edema in animals. Strikingly, the inhibitory effects of betulin, betulinic acid, and UA in carrageenan and serotonin paw edema tests could be blocked by progesterone, actinomycin D, and cycloheximide (54) suggesting that the anti-inflammatory actions of these PTs are mediated by mechanisms related to glucorticoid receptor activation and mRNA and protein biosynthesis. UA inhibited phorbol ester induced edema of mouse ears (57, 58). The anti-inflammatory activity in this model seems to be common to many PTs (including α- and β-amyrin, UA, OA, hederagenin, GA, lupeol, lupeol-3-acetate, betulin, betulinic acid, and their sapopins), which were effective with comparable ED₅₀ values in the range from 0.1 to 1.3 μmol per ear (59, 60). Topical applications of dihemiphthalate derivatives of the oleanane series, but not GA and deoxyglycyrrhetol, reduced arachidonic acid-induced ear edema in mice in parallel to a decreased PGE₂ and LTB₄ formation with ID₅₀ values of about 2 mg per ear (61). Inhibition of dextran induced leukocyte migration into the pleural space by GA (32) and by a crude BA containing extract from B. serrata gum resin (65) were reported.

Fig. 3

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>lupeol</td>
<td>CH₃</td>
</tr>
<tr>
<td>betulin</td>
<td>CH₂OH</td>
</tr>
<tr>
<td>betulinic acid</td>
<td>COOH</td>
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</table>
Immunomodulation

Saikogenin D, as well as saikosaponin a and d, augmented non-specific protection in mice against Pseudomonas aeruginosa infection in terms of increased survival (63). Soyasapogenol E (Fig. 4) was demonstrated to inhibit TPA induced Epstein-Barr virus early antigen activation (64). A crude ethanolic extract of B. carterii gum resin decreased mortality in mice to which a suspension of E. coli had been administered intraperitoneally (65). This in vivo observation was suggested to be due to stimulated phagocytic activity by the lipophilic non-saponifiable fraction. A nonstandardized BA containing extract displayed either immunosuppressive or immunomodulating actions in mice depending on the dosage schedules and timings of administration in relation to antigen stimulation, whereas a preincubation of peritoneal macrophages at concentrations from 15 to 62 µg/ml stimulated the phagocytic activity in vitro (66).

Clinical Studies

The GA derivative carbenoxolone has been used as an anti-ulcerative drug (for monographs see 67, 68). In Japan, a preparation of GL combined with glycine and cysteine has been widely used as an anithepatitis drug. Hematological and histological improvement following commencement of GL has been reported in patients with chronic hepatitis (for references see 12). Since GL was observed to inhibit virus growth and to promote inactivation of virus particles (40), it was also used for the prevention of recurrence of hepatitis B after liver transplantation (69). However, the therapeutic index of GA and derivatives is limited due to potent mineralocorticoid actions, which are mediated by interaction with steroid metabolism. OA has been marketed in China as an oral drug for human liver disorders and was reported to have been used successfully (for review see 70). Promising preliminary results have been obtained with BA containing preparation in first clinical studies. In line with the in vitro observations, p. o. application of a commercial crude chloroform/methanol extract from the B. serrata resin with a nonstandardized content on BAs inhibited increased urinary cysteinyl-LT excretion in astrocytoma patients and reduced peritumoral brain edema volumes (71). In a prospective, controlled clinical study, a 7-day treatment of patients with malignant glioma resulted in a significant reduction of perifocal edema, but no reduction of tumor sizes (72). The 5-LO inhibition is proposed to be of pharmacotherapeutic value in brain tumor patients with increased LT biosynthesis. In some clinical studies (open and placebo-controlled) the use of the commercial chloroform/methanol B. serrata gum resin extract yielded promising results in a limited number of patients with chronic polyarthritis (73, 74). A nonstandardized, powdered preparation from the gum resin of B. serrata, which contained BAs, was as effective as sulfasalazine in treating ulcerative colitis as observed in an open, non-randomized clinical trial (75). Since these observations are based on the results of pilot studies with few patients (14 to 39), data from state-of-the-art clinical trials with standardized BA preparations and a greater number of patients are needed to establish the probable value of BA containing drugs for the treatment of severe human diseases.

Summary and Perspectives

There are many reports on biological actions of PTs, which could be relevant for pharmacological effects including their anti-inflammatory properties. Data from in vitro studies could help to understand the mechanisms of their actions. It is, however, remarkable that many observations reported were obtained in studies using exorbitantly high concentrations. Because of the limited solubility and nonselective actions of high concentrations of lipophilic compounds the interpretation of the results must be critically reconsidered in order to evaluate the practical implications of a probable use in vivo. In summary, we suggest that in vitro effects produced with concentrations much greater than 10 µM will hardly have a practical relevance. But there are reports also on highly interesting actions for a limited number of PTs at reasonable concentrations in vitro, which may help to explain pharmacological actions and side effects in vivo. In general, however, the current knowledge on effects of PTs and structure-activity relationships is preliminary. In conclusion, there exists promising data suggesting that PTs are a class of compounds with highly interesting biological effects worth to be studied more carefully. The low acute toxicity of these natural compounds and hints from SAR studies, which suggest that defined substituents on the lipophilic 5-ring backbone can increase selectivity and potency of a desired action, indicate that PTs might provide a rich natural resource of lead compounds for drug development.

References
