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Invited review

Discovery of new anti-inflammatory drugs from plant origin

C.J. Beukelman, E. van den Worm, C. Henriette, Q. van Ufford, B.H. Kroes, A.J.J. van den Berg

SUMMARY

From traditional systems of medicine like Ayurveda and Traditional Chinese Medicine, herbs with (assumed) anti-inflammatory properties are taken into study in order to isolate the active principles. The isolation is guided by biological activity in relevant bioassays. Here the background of several bioassays is discussed and some examples are presented, with special attention to apocynin, a lead compound intensively studied by our research group as well as in close co-operation with several other institutes.

Key words: inflammation, herbal medicines, apocynin, experimental colitis

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INTRODUCTION

Despite all the successes of scientifically-based medicine and the constant optimisation of drug use in our civilised and highly developed society we are still in need of new remedies. One of the principle aims of medicinal plant research is the discovery of new biologically active leads in areas of unmet medical need. In our research we focus on constituents in plant extracts and herbal preparations, which are responsible for the biological activity, so-called biomarkers.[1,2] Since it has become clear that immunological derangements are often involved in the aetiology of many diseases, chronic inflammatory disorders in particular, we have a special interest in immunomodulatory plant constituents. During the last twenty years our research strategy has been based on the activity-guided isolation and subsequent identification of active principles from complex plant extracts.

Traditional systems of medicine like Ayurveda and Traditional Chinese Medicine have made use of many medicinal plants for millennia. From these systems, herbs with (assumed) anti-inflammatory properties still in use today, are selected and taken into study. From these plants extracts are prepared and tested for activity in our bioassays, e.g. assays for human complement activity or T-cell proliferation, or the chemiluminescence assay. Most active extracts are fractionated (e.g. by column chromatography) and the fractions obtained further purified on guidance of biological activity, eventually resulting in homogeneous single active principle(s), see figure 1. Subsequently, these lead compounds are subjected to structure elucidation using state-of-the-art spectroscopic methods (mass spectroscopy, nuclear magnetic resonance, and infrared spectroscopy). In order to obtain molecules with improved qualities, structure activity relationship (SAR) studies are conducted with derivatives obtained by straight-on chemical modifications of the original leads. In parallel with drug optimisation through SAR studies, attention is also paid to the unravelling of working mechanisms. Finally, we aim at in vivo proof of principle. In this paper, the background of several bioassays is discussed and some examples are presented, with special attention to apocynin, a lead compound intensively studied by our research group as well as in close co-operation with several other institutes.

The Human Complement System

The complement system is part of our innate (non-

Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Utrecht University, P.O. box 80082, 3508 TB Utrecht, The Netherlands

Author for correspondence:

C.J. Beukelman, Dept of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Utrecht University, P.O. box 80082, 3508 TB Utrecht, The Netherlands

adaptive) humoral immune system and plays an important role in our defence mechanism. It consists of more than 30 proteins. Next to its beneficial effects, complement may give rise to tissue damage in many situations, including mechanical injury, formation of immune complexes caused by (auto) antibodies, reperfusion damage, and contact with exogenous materials such as prostheses or renal dialysis membranes. In our research program we aim at the isolation of plant constituents, which interfere with complement activity.

Freshly prepared serum from human volunteers is taken as complement source. Sheep red blood cells serve as target cells for classical pathway whereas rabbit red blood cells are used for determination of alternative pathway activity.[3] For interference with individual complement components, selectively depleted reagents are produced.[4]

Liquorice (Glycyrrhiza glabra) is taken here as example. Its anti-inflammatory as well as anti-allergic activities have been attributed to a mixture of closely related main constituents, glycyrrhizin. We found that the aglycone b-glycyrrhetinic acid is a potent inhibitor of the classical complement pathway activity (IC50= 35 μM), whereas no inhibitory effect was observed towards the alternative pathway (IC50> 2500 μM). In addition, it was found that the activity was conformation-dependent since the a-form did not show any effect. Detailed mechanistic studies revealed that b-glycyrrhetinic acid acts at the level of complement component C2.

T-cell proliferation

T-cells play a most important role in the regulation of immunity and are therefore a major target of immune intervention. Many drugs have been developed over the last decades, including corticosteroids, cyclophosphamides, methotrexate, cyclosporine A and others. However, the clinical use of these immunosuppressants is hampered by side effects, the major disadvantage being their toxicity. In this perspective we screen plant extracts for their ability to inhibit T-cell proliferation.

Peripheral blood lymphocytes were obtained from buffycoat residues using Ficoll-Hypaque centrifugation, according to manufacturer's instructions. Under sterile conditions cells were diluted to 2.10^6 cells per ml medium (RPMI 1640 supplemented with 10% foetal calf serum), dispensed in 96-wells microtiter plates (50 μl/well), activated with phytohaemagglutinin (PHA; 0.1 mg/ml) and incubated at 37°C with 50 μl of test samples in appropriate dilution ranges for four days. T-cell proliferation was determined using a modified colorimetric MTT assay.

Picrorhiza scrophulariiflora and Picrorhiza kurroa are extensively used in traditional medicine in China, Tibet, Nepal, India, and Sri Lanka for the treatment of various immune-related diseases. Both plants are used for asthma, jaundice, and arthritis, which are T-cell mediated diseases. Two curcubitacins, named deacetylpicracin and picracin, were isolated from the roots of P. Scrophulariiflora.[5] Both compounds were identified as aglycones of known 11-deoxycurcubitacin glucosides, and exhibited dose dependent inhibition of PHA-induced T-cell proliferation; IC50 values were determined to be 1 μM.

Chemiluminescence

Neutrophils, monocytes and macrophages are phagocytic cells, which act primarily by engulfing and digesting bacteria, cellular debris and other particulate matter. These specialized guardians are essential for our health, and play an important role in inflammation processes. Under certain conditions, however, they escape from regular control, which may lead to chronic inflammatory conditions, such as rheumatoid arthritis and colitis. We study effects of plant-derived compounds on functional characteristics of human phagocytic cells and determine in this respect their oxygen consumption, production of reactive oxygen metabolites, and cytokine production. Anti-oxidant activities of plant constituents may also be relevant in the treatment of chronic inflammation and are determined as well.

Polymorphonuclear leukocytes (PMNs) were isolated from buffycoat residues and diluted to 1.10^7 PMNs per ml Hank's balanced salt solution (HBSS) and dispensed in white 96-well flat-bottom microtiter plates in 50-μl amounts. Subsequently, 50 μl of an appropriate dilution range of extract and 50 μl of luminol (0.1 mM) were added to each well. The cells were activated with 50 μl of serum-treated zymosan (0.8 mg/ml), after which the luminescence of each well was monitored every two minutes for 30 minutes, in a Titertek Luminoscan luminometer. Maximum peak levels were used for the calculations. Controls consisted of cells with luminol and buffer.

Simons et al[6] subjected P. kurroa to an activity-guided isolation procedure, with the chemiluminescence mentioned above as readout system, and discovered that one of its constituents, apocynin, selectively inhibited the production of the reactive oxygen species (ROS) by activated human PMNs.

Apocynin and inflammatory disorders

Apocynin, 4'-hydroxy-3'methoxy-acetophenone, was discovered as plant constituent many years ago.[7] and oc-

curs in several plant species, including Picrorhiza kurroa, a small perennial herb growing at high altitudes in the Himalayas and used traditionally in Ayurvedic medicine in India and Sri Lanka.[8]

Apocynin proved to be a potent inhibitor of the assemblage of NADPH oxidase. Interestingly, apocynin does not seem to interfere with other defence mechanisms of the PMN, as it does not affect phagocytosis or intracellular killing.[9] For this reason apocynin has become an important, widely used, experimental tool to block NADPH oxidase activity. Apocynin is also explored for activity in several in vitro, ex vivo and experimental animal models amongst which models for arthritis and colitis.

Experimental Arthritis

Collagen type II-immunized rats were treated with different doses of apocynin in the drinking water (0.3 - 200 mg/mL) starting 9 days after immunization (this is just before the onset of arthritis, but after development of a specific immune response).[10,11] Treatment was terminated 14 days later, at the time when joint swelling in the control group was maximal. Surprisingly, the lowest apocynin concentration protected the animals from joint swelling, whereas increasing the dose up to 200 mg/mL did not improve the effect. Even 100 days after immunization, no flare-up of the joint swelling was observed in apocynin-treated rats. Treatment of rats with low doses of apocynin also reduced plasma IL-6 levels. Interestingly, it was demonstrated that the severity of collagen-induced arthritis correlates with increased IL-6 production.[12]

Another effect of apocynin, which may emphasize its importance in the treatment of rheumatoid arthritis (RA), is that apocynin inhibits inflammation-mediated cartilage destruction in human articular cartilage explants, without having adverse effects on the cartilage itself[13]. In these experiments, apocynin was added to cultured peripheral blood mononuclear cells of RA patients. Cartilage-destructive activity was determined after addition of culture supernatant to tissue samples of the cartilage explants.

Experimental colitis

Palmen et al examined the effects of apocynin in acute and relapsing experimental colitis in rats.[14] Acute colitis was induced by intra-colonic administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) in 30% ethanol (30 mg in 0.25 mL). Relapsing colitis was induced by a subcutaneous injection of TNBS, 5 weeks after the induction of acute colitis. In both acute and relapsing colitis models, the animals received two intravenous injections of apocynin (4 mg/kg bodyweight) at day 0 and 3 (acute) or day 35 and 38 (relapsing). After sacrificing the rats, the influx of macrophages and PMNs into colon tissue as well as MPO activity and macroscopical damage scores of the colon were determined. In the model for acute colitis, apocynin significantly reduced the damage score, MPO activity, and the number of macrophages and PMNs in the colon. Apocynin-treatment in relapsing colitis resulted in a striking improvement of the damage score to almost normal values, significantly lower MPO-activity, and decreased numbers of colonic macrophages. These experiments show that, besides inhibition of ROS production, apocynin may also prevent tissue damage in IBD by inhibiting the influx of inflammatory cells into the colon.

Rachmilewitz et al[15] tested apocynin in a rat model for Crohn's disease, which represents inflammation and damage in the small intestine. They showed that apocynin in the drinking water resulted in an effective decrease in the extent and severity of jejunal damage, that the villi of the jejunal wall were almost normal, while no granulomas were observed in any of the rats.

In conclusion

Based on the positive effects on the pathogenesis of inflammation of both the small and large intestine by apocynin as mentioned above, a series a apocynin-derived molecules have now been synthesized in our laboratory and are currently tested in the chemiluminescence assay. Apocynin congeners with most promising inhibitory activities in this respect will be tested in animal models in the near future. Keeping in mind the existence of probably 500,000 higher plants, it will be clear that the plant kingdom is a rich source of molecules with most interesting biological activities; many of them may serve as lead compound in drug development. Natural products are already evolutionary selected;[16] what is needed is their optimization and processing into useful drugs.

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