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REVIEW

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Low-grade chronic inflammation and transcriptomics: how molecular pharmacognosy can help find new natural treatment alternatives—a narrative review

Massimo Fioranelli[1], Maria Grazia Roccia[1], Bianca Przybylek[2], Francesca Romana Sconci[2] and Maria Luisa Garo[3*]

Abstract

Background The inflammatory response is fundamental to the maintenance of an organism's physiological homeostasis. Inflammation is controlled by a series of biological events driven by specific inflammatory molecules. When inflammation is within the homeostatic range, it is considered physiological; however, it becomes pathological when it exceeds the immune system's homeostatic control.

Main text Nowadays, the treatment of chronic pathological inflammation is a challenge for pharmacology, as current anti-inflammatory drugs are intended to control acute inflammation. The aim of this narrative review was to provide an overview of the role of molecular pharmacognosy and to demonstrate how current transcriptomics techniques can make an important contribution to the study of the biological functions of natural products in the context of multicomponent/multitarget medication. From our findings, although very few studies have been identified, encouraging results for low-grade chronic inflammations (LGCIs) of various causes emerged in recent transcriptomic studies on multicomponent medicinal products composed of plant and organ extracts at the level of the skin and the musculoskeletal system (Traumeel: Tr14), the liver (Lycopodium compositum: HC-24), and the joints (Zeel-T: Ze-14).

Conclusion For adequate control of LGCI, molecular pharmacognosy may be an effective approach to exploring potentially useful herbal agents that are consistent with both physiotherapeutic tradition and modern pharmacology.

Keywords Low-grade chronic inflammation, Systems medicine, Low-dose medicine, Molecular pharmacognosy, Phytocomplexes, Multicomponent/multitarget medications

1 Background

Inflammation is a biological process that ranges from the accumulation of leukocytes to the release of various types of lipid mediators, such as eicosanoids, and proteins, such as cytokines and chemokines, aimed at containing the reaction of the affected site, initiating the immune response, eliminating the triggering factors, and restoring the tissue's integrity and functionality [1]. To some degree, inflammation represents the physiological response of the immune system to noxious stimuli [2]. When this pro-inflammatory response exceeds the

*Correspondence:
Maria Luisa Garo
marilu.garo@gmail.com
[1] Department of Human Sciences, Guglielmo Marconi University, Via Plinio 44, 00193 Rome, Italy
[2] Istituto Terapie Sistemiche Integrate, Casa di Cura Villa del Rosario, Via Flaminia 449, 00181 Rome, Italy
[3] Istituto Terapie Sistemiche Integrate, Casa di Cura Sanatrix, Via di Trasone, 6, 00199 Rome, Italy

immune system's control capacity, the increasing inflammatory response leads to the appearance of inflammatory symptoms and thus to the progression of inflammation from a physiological to a pathological state [3].

In some cases, the inflammatory process thus activated does not completely subside and does not restore the integrity and functionality of the tissue, but remains in a kind of pathological latency phase known as low-grade chronic inflammation (LGCI) [4], which can promote a number of chronic diseases in the medium term, such as metabolic syndrome, non-alcoholic fatty liver disease (NFALD), type 2 diabetes mellitus, and cardiovascular disease [5], and increases the risk of all-cause mortality in an apparently healthy adult general population [6]. The pathological profile of LGCI is characterized by a specific timing in which the two classical inflammatory phases of maintenance and recovery coexist, the inflammation process is continuously activated, and the phase of *restitutio ad integrum* is not completed. It is also characterized by a specific altered chronobiology of the sequential release of cytokines [7]: Interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α),) and interleukin 6 (IL-6) are not effectively downregulated after the initial phases of the inflammatory response, and inflammatory expression is consistently 3–4 times higher than baseline without upregulation of the anti-inflammatory IL-10 (Fig. 1).

2 Main text

In recent years, systems medicine (SM) has emerged as an interdisciplinary field that takes an integrative and holistic approach to the systems-level study of the human body to improve the understanding, prevention, and treatment of complex diseases [8, 9]. In the context of chronic inflammation, it has been proposed as an appropriate tool for understanding and managing various phenomena that are otherwise not easily understood with an organ-oriented approach [10]. In this new approach, in which the human body is considered as a network system with the capacity for self-regulation through the dynamic interactions between the different networks, SM assumes that the human organism can be considered a network composed of neuroendocrine, immune-inflammatory, metabolic, and energetic cellular networks [11], and characterized by a strong capacity for self-organization thanks to the mechanisms of self-regulation that ensure intrinsic stability, robustness, and resilience. In this approach, any pathological phenomenon can be considered a progressive dysregulation of networks that can be counteracted using a bioregulatory systems medicine (BrSM) approach [12, 13], that is, an approach in which the SM can be combined with pharmacological interventions based on low-dose multicomponent/multitarget drugs that act simultaneously and precisely on multiple networks.

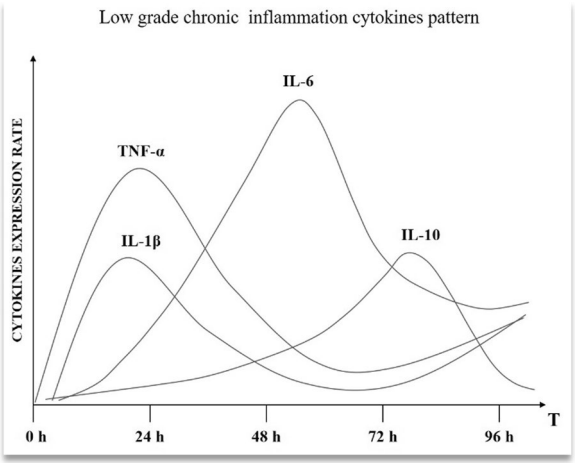

Fig. 1 Chronobiologic expression of cytokines in the presence of low-grade chronic inflammation. IL-6 (interleukin 6), IL-1β (interleukin-1 beta), IL-10 (interleukin 10), and TNF-α (tumor necrosis factor alpha). *Source*: Fioranelli, M., Roccia, M. G., Flavin, D., & Cota, L. (2021). "Regulation of inflammatory reaction in health and disease". International Journal of Molecular Sciences, 22(10), 5277

Because the regulation of networks relies on the transport of messenger molecules (i.e., cytokines, hormones, neuropeptides, and growth factors) acting in a range of physiological sub-nanomolar concentrations, the investigation of the potential therapeutic application of low-dose signaling molecules should be advanced through an approach of low-dose pharmacological intervention [10], which allows drugs to act directly on networks of low-dose signaling molecules so that physiological responses are controlled by endogenous signaling molecules. A commentary by Bernasconi et al. assumes the possibility of using a potential clinical therapeutic low-dose medicine (LDM) to restore network homeostasis by stimulating a pathologically impaired (downregulated) cellular signaling pathway with the same cytokines, hormones, neuropeptides, or growth factors that are physiologically involved in cellular signaling, and using antagonistic molecules to rebalance the levels of the pathologically upregulated molecules using negative feedback mechanisms [14]. The aim of this review is to provide an overview of the role of molecular pharmacognosy and to show how current transcriptomics techniques can make an important contribution to the study of the biological functions of natural products in the context of multicomponent/multitarget medication.

2.1 Molecular pharmacognosy

Interesting contributions to LDM can be supported by molecular pharmacognosy [15], a new intermediate science, formally defined by Dr Lu-qi Huang (Professor

of Pharmacognosy at the Institute of Chinese Materia Medica) in 1995 [16], that is, the combination of pharmacognosy, the science of drugs of natural origins, and molecular biology dealing with the study of classification, identification, cultivation, and protection of crude drugs at the genetic level with a theoretical and methodological basis on molecular biology [16].

The most important source of natural active ingredients is the plant kingdom [17], an impressive biochemical factory capable of synthesizing a range of active ingredients in high quality and quantity, characterized by scaffolding and structural heterogeneity and greater rigidity than synthetic molecules [18]. These properties underline that natural active ingredients have been optimized by evolution to exert specific biological functions, such as the modulation of endogenous defense mechanisms. Although 85% of modern active substances come from natural sources, the use of herbal plants has been relegated for decades to the niche of "traditional use." Nevertheless, the advance of genomics, proteomics, metabolomics, and other "omics" disciplines has been significantly contributing in understanding the qualitative/quantitative characterization of herbal plant constituents, thus creating a virtuous circle of research as depicted in Fig. 2.

Although the application of high-throughput screening (HTS) has reached its maximum potential in identifying natural active ingredients, it has also revealed its limitations when it comes to finding new active ingredients that meet the requirements of complex non-natural origin medicines [19, 20].

To overcome these limitations, researchers can use innovative technological platforms for the discovery and evaluation of natural products, such as a database of 2D/3D structures of compounds of pharmacological interest, a HTS database, a database of known biological targets, an ethnopharmacological database, and a virtual screening environment.

With these tools, modern molecular pharmacognosy is validated as a new and solid database for the study of the biological activities of herbal agents, alone or in combination with phytocomplexes.

2.2 Advancements in the study of natural products

Of the approximately 250,000 available species of higher plants, only 15% have been subjected to phytochemical screening, and about 6% have been examined for their biological properties [21]. The process of discovery and development of natural products begins with the selection of plants, followed by the extraction and isolation of the pure extract, their biological screening and synthesis [22, 23]. Over the decades, various molecular biology techniques have been applied in pharmacognosy to identify new medicinal plants [15]. The possibility of identifying new natural products is complicated by the complex regulation of the biosynthesis of these products depending on the environment, so that the conditions under which the producing organisms are cultivated can have a

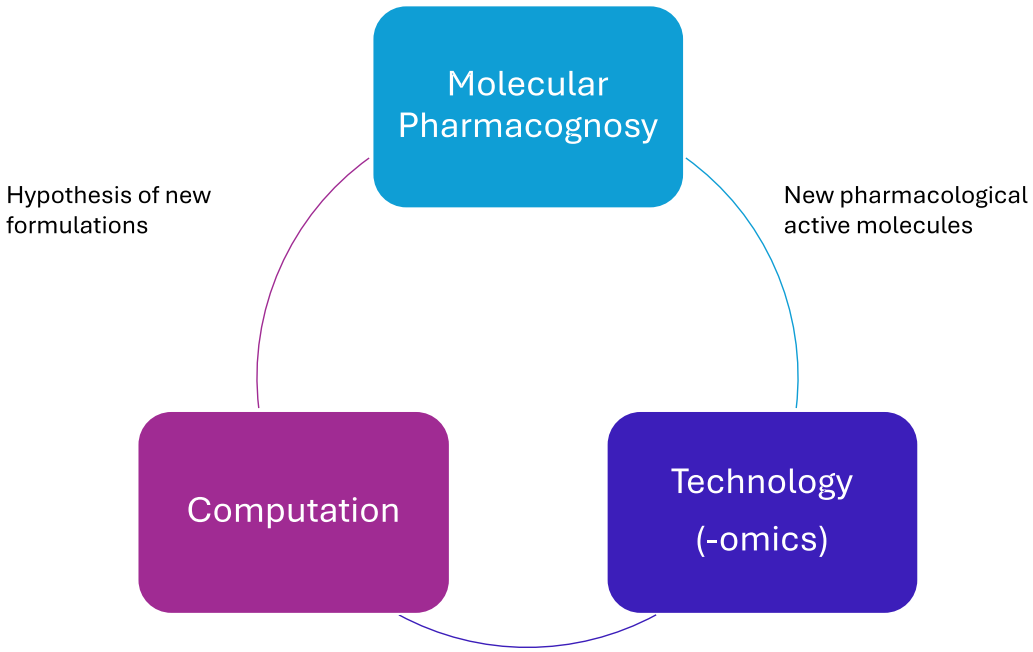

Fig. 2 Virtuous circle of research in molecular pharmacology

significant impact on this possibility [24]. In the extraction and isolation of natural products, biological activity-guided fractionation, and isolation methods are generally used in conjunction with chromatographic separation techniques [22]. In contrast to other methods where the plant extract is separated stepwise, biological activity-guided fractionation allows all fractions to be analyzed for biological activity, and only those fractions that exhibit significant activity are selected; the process continues until the pure isolate responsible for the targeted biological activity is identified [22, 25]. In this way, chemical characterization and structural elucidation are performed only after the active isolates have been identified [22]. In this context, spectroscopic analysis is the most important approach for the structural determination of phytochemicals. After a preliminary biological screening of the extracts, the bioactive extracts can be quickly fractionated through high-performance liquid chromatography. Subsequently, the chemical characterization of the fractions is performed using high-resolution mass spectrometry (HRMS), liquid chromatography-mass spectrometry (LC–MS),) and nuclear magnetic resonance spectroscopy (NMR) [24, 26, 27]. NMR spectroscopy is a simple and reproducible method that provides direct quantitative information and detailed structural information, but with a relatively low sensitivity, so that usually only profiles of the most important components can be created. HRMS is the gold standard for qualitative and quantitative metabolite profiling and is most commonly used in combination with LC. The application of these techniques makes it possible to shorten the entire process from extraction to isolation of pure natural products from months, as was the case a few years ago, to a few days [22].

Bioactivity-guided isolation is a laborious process with a number of limitations, but various strategies and technologies have been developed, such as the creation of libraries compatible with HTS, so that crude extracts can be pre-fractionated into subfractions that are more suitable for automated liquid processing systems [24]. In recent years, various technological and scientific improvements, complemented by computational tools, have opened up new opportunities for drug discovery from natural productsproduct [28].

According to recent studies, artificial intelligence and machine learning have contributed to the discovery of bioactive natural products and the development of drugs based on natural products, enabling the identification of a large number of molecules from nature. Applications of machine learning in genome mining and dereplication techniques have reduced the effort required to obtain natural crude extracts and accelerated the discovery of new natural chemical products. The application of dimensionality reduction techniques, such as principal component analysis, t-distributed stochastic neighbor, or, recently, tree-dimensional trees, has have helped to compare the properties of natural products with those of drugs and synthetic libraries. Regression and classification approaches with machine learning features allow the prediction of the biological activities or properties of such natural products, although new research is required to overcome some limitations due to the imperfect ability of machine learning to avoid the accidental discovery of valuable bioactive natural products [29, 30].

2.3 Multicomponent/multitarget BrSM drugs and phytocomplexes

To comprehend how herbal agents can respond to the low-grade chronic inflammatory state, it is important to understand plant biosynthesis, which in turn is the basis for multicomponent/multitarget bioregulatory system medicine.

Each plant produces two basic groups of substances: primary and secondary metabolites. The former carries out metabolic activities associated with the maintenance of the plant's life, whereas the latter are mainly used to implement adaptation strategies and defend the plant against environmental aggressors such as bacteria, fungi, viruses, predators, and so on [31], and are essential for its survival in the environment. These secondary metabolites maintain plant coexistence and coevolution [32]. Coevolution is crucial in explaining why certain substances of plant origin exhibit biological activity in animal organisms. Indeed, many biologically active molecules have been conserved (or modified to a limited extent) across evolutionary phases, starting with the emergence of unicellular organisms. Substances such as GABA or acetylcholine are present in the animal nervous system as well as in *Valeriana officinalis* L. [33]; an interleukin important for inflammatory processes such as IL-12 is also present in starfish [34] in almost identical form as in mammals.

Traditionally, pharmacological research has focused on the isolation and subsequent synthesis of single molecules with well-defined biological effects, neglecting phytocomplexes in their entirety, which, however, could potentially better address the pharmacological needs of modern SM. On the contrary, in the research for BrSM multicomponent/multitarget drugs, phytocomplexes play a crucial role. A phytocomplex generally has a more nonspecific effect with a broader spectrum, and the components of the phytocomplex can exert biological effects in synergy. It is not uncommon for a phytocomplex to have different, valuable, and improvable pharmacological properties than the individual chemical constituents. From the point of view of pharmacological mechanisms,

the action of phytocomplexes, although based on mechanisms also common to synthetic medicines, differs from them in that it is essentially polyvalent. The simultaneous presence of different compounds with different chemical properties influences the pharmacodynamic and pharmacokinetic properties of the active ingredients and modulates their biological action [35].

For example, *Matricaria chamomilla* L. [36, 37] is composed of matricin, a component of the phytocomplex of Chamomilla, converted in the environment of gastric acid into chamazulene carboxylic acid, a molecule with anti-inflammatory effects (downregulation of COX-2 signaling). Furthermore, the flavonoids contained in the phytocomplex enhance the anti-inflammatory effect of chamazulene carboxylic acid, whereas the compound bisabol, which comes from chamomilla, also exerts a stomach-protecting effect. Therefore, a phytocomplex like this can be considered pharmacologically the smallest pharmacological multicomponent/multidrug unit. This is because the components of the phytocomplex are much easier to link with other active phytocomplexes and ingredients, both natural and synthetic, than single molecules with a robust, specific effect.

2.4 Transcriptomics for the study of natural multicomponent/multitarget BrSM medication activity

To date, to investigate the possible effects of multicomponent/multitarget drugs containing phytocomplexes and other compounds of natural origin, molecular pharmacognosy has been focused on transcriptomics, the study of the "transcriptome," a term now widely understood to mean the complete set of all the ribonucleic acid (RNA) molecules (called transcripts) expressed in some given entity, such as a cell, tissue, or organism [38]. Analyzing the transcribed RNA starting from the genotype at a specific time point and under specific stimulation conditions, transcriptomics aims to establish a link between the genome, the proteome, and the cellular phenotype, describing the flow of information from DNA to RNA to proteins. The synthesis of RNA on a DNA template is identified with the transcription process, and this step is the most important for the regulation of gene expression in living organisms. By analyzing the entire RNA of a cell, i.e., its transcriptome, cell physiology can be studied under specific experimental conditions chosen to simulate a particular environment and condition.

Encouraging results for LGCIs of various causes emerged in recent transcriptomic studies on multicomponent medicinal products composed of plant and organ extracts at the level of the skin and the musculoskeletal system (Traumeel: Tr14) [39, 40], the liver (Lycopodium compositum: HC-24) [41], and the joints (Zeel-T: Ze-14) [42].

From the gene-level studies designed and conducted by St. Laurent III et al., it appears that Tr14 can positively influence the expression of gene classes involved in tissue damage repair processes, creating more favorable conditions for efficient physiological resolution of the inflammatory phenomenon and equally efficient triggering of functional recovery of the damaged tissue. In particular, the negative and positive modulation of IL-1β and IL-36 expression draw a favorable picture for the resolution of the inflammatory process [39, 40]. The most important finding of the study is the demonstrated ability of Tr14 to profoundly influence the inflammatory response. The results illustrate how Tr14 crucially modulates the entire inflammatory process from its initial stages by maintaining the reactivity required to initiate the process and balancing the duality between inflammation and repair as the response develops. Analysis of the data from the experiments with Tr14 and diclofenac confirms the modulatory effect of inflammation and highlights the ability of Tr14 to promote the pro-resolution phase. Transcriptomics shows a strong correlation between the chronobiology of the inflammatory phenomenon and the effect of Tr14. In a transcriptomic in vivo study in a mouse model of NFALD aimed at testing the ability of HC-24 to improve cholesterol homeostasis and reduce inflammation in the liver, Mueller et al. demonstrated how HC-24 effectively controlled liver inflammation and could reduce the free cholesterol fraction, preventing the accumulation of cholesterol in the liver and the development of resulting inflammation and organic insufficiency [41].

A recent transcriptomic study conducted on Ze-14, a multicomponent medicinal product composed of generally well-tolerated plant and organ extracts, already proved to reduce symptoms of osteoarthritis, including stiffness and pain, with a good safety profile [43, 44]; it appears that Ze-14 can positively influence the cellular metabolism of chondrocytes from primaries suffering from osteoarthritis by promoting chondrogenesis. Type II collagen synthesis is essential for the maintenance of the good morpho-functional properties of cartilage. Apart from the inherent complexity of genomics and proteomics studies, the ex vivo model chosen to study the activity of Ze-14/ZEEL® T proved to be particularly valid and provides data of particular interest on the profound effect of Ze-14/ZEEL® T on cartilage cell metabolism. This study underlines the multicomponent/multitarget character of Ze-14/ZEEL® T: the duality between inflammatory and matrix synthesis processes becomes as clear as the drug's ability to modulate both aspects in a profound and balanced manner. From a SM perspective, it is also fascinating how the ability of Ze-14/ZEEL® T to

reduce imbalanced tryptophan metabolism toward the kynurenine pathways can have a systemic effect in addition to a local anti-inflammatory effect [42].

3 Conclusion

In conclusion, bioregulatory system medicine can provide the pharmacological field with new and interesting developments. Associating SM with molecular pharmacognosy all its omics fields can contribute significantly to the investigation of the actual biological functions, especially those that are at the base of many low-inflammatory phenomena, and to the identification of anti-inflammatory formulations containing multiple phytocomplexes and/or other active molecules of natural origin. New insights are required to fully comprehend the effects of multicomponent/multitarget drugs. Future research could be focused on the possible role of natural compounds and their attitude toward controlling the phenomenon of inflammation, thus opening the way to better treatment of chronic inflammatory diseases where the permanent use of conventional anti-inflammatory drugs is impossible.

Abbreviations

BrSM	Bioregulatory systems medicine
IL-6	Interleukin 6
IL-10	Interleukin 10
IL-1β	Interleukin-1 beta
LGCI	Low-grade chronic inflammation
SM	Systems medicine
LDM	Low-dose medicine
TNF-α	Tumor necrosis factor alpha
Tr14	Traumeel
Ze14	Zeel T

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Author contributions

MF: Conceptualization, methodology, supervision. MGR: Visualization, investigation and writing—original draft preparation. BP: Validation and writing—reviewing and editing: FRS: Software, validation and writing—reviewing and editing. MLG: Data curation, writing—original draft preparation.

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Competing interests

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