Chapter

Medical Implications of the Relationships among Protein Denaturation, Necrosis and Inflammation: An Intriguing Story

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Abstract

This story deals with the role of protein denaturation in inflammation. The starting point was the description of the necrotizing action of inflammatory proteins, followed by the discovery of the antidenaturant action of NSAIDs (nonsteroidal anti-inflammatory drugs). Hence, the idea is that the antidenaturant action accounted for the action of NSAIDs. This hypothesis was dropped following the discovery of the antiprostaglandin action of NSAIDs, which shifted the focus to the arachidonic acid cascade. It was revived by assuming that protein denaturation is a process in its own, suitable for separate medical treatment. This approach led to bendazac and bindarit, the first selective antidenaturant drugs. This experience shows that protein denaturation has two main pathological sequelae. The first concerns the so-called primary (innate) inflammation. The second sequela concerns the so-called secondary (acquired) inflammation. Natural antidenaturant agents represent a promising alternative to the synthetics bendazac and bindarit. Within this framework, tendinitis finds a separate but significant place.

Keywords: inflammation, protein denaturation, bendazac, bindarit, fatty acids, tendons

1. Introduction

To start with, inflammation is part of homeostasis, the fundamental process of life of preserving the milieu intérieur by reacting to changes with opposing measures [1–3]. More specifically, inflammation is "the vital response to injury", which may be related to exposure to infection, toxins, damaged cells, waste, and chemical and physical agents [4, 5]. The function of inflammation is to repair the damage that does not result from the inflammation itself, but from overactivation or deviation of the underlying physiological process. Consider the five cardinal signs of inflammation described by Celsius and Galen over 2000 years ago: heat (calor), pain (dolor), redness (rubor), swelling (tumor), and impairment (functio laesa). Without pain, for example, a person would be exposed to disease without realizing it. Similarly, impairment (functio laesa) implies

the setting aside of a function to allow recovery. A fracture would not be repaired without temporary immobilization of the broken bone. The overlap of physiological and pathological elements is the key point of the medical treatment of inflammation. Based on these introductory remarks, there are two inflammatory processes. The innate one is based on the hyperactivation of genetically innate physiological processes. Acquired inflammation is different in that it involves the immune system.

2. Pathophysiological considerations

It consists of an immune reaction adapted to each aggressive agent so as to neutralize it without damaging the surrounding tissues. This measure takes into account the genetic characteristics that distinguish each living being, as well as the chemical structure and macromolecules that the organism must take up. Acquired inflammation combines the collateral damage of innate and acquired processes. For example, deaths from SARS-CoV-2 and variants are caused not only by the virus, which penetrates and kills cells but also by the excessive immune-inflammatory reaction that impairs respiration and blood clotting.

With this in mind, the current story begins about 60 years ago with the description of the irritating and necrotizing effects on the skin of protein complexes related to both acquired and innate inflammation [6–9]. It should be noted that a similar necrotizing effect is produced by urea, a metabolite of the aforementioned complexes [10]. Subsequently, NSAIDs were found to exert an antidenaturant action at concentrations that roughly correspond to their anti-inflammatory effects [11, 12]. The resulting hypothesis was that "most strongly anti-inflammatory drugs might owe at least part of their effects on some biochemical processes to their physicochemical property of interacting with proteins." The quoted portion is from the original article [12]. Simply put, the hypothesis was that the antidenaturant effect of NSAIDs underlies their mechanism of action. Hence, the protein denaturation assay was proposed as a simple screening test for antirheumatic and antiphlogistic drugs [13, 14]. The protein-centered hypothesis had some inconsistencies. For example, the antidenaturant effect occurs at relatively higher concentrations than at anti-inflammatory concentrations [15]. In addition, antidenaturant drugs may not have anti-inflammatory effects [16].

Inconsistencies aside, the above hypothesis was abandoned following the discovery of the antiprostaglandin action of aspirin-like drugs, which shifted the focus to the arachidonic acid cascade [17]. Prostaglandins are lipid autacoids derived from arachidonic acid that have widespread physiological roles in the body, including homeostatic functions involved in the inflammatory process [18]. The arachidonic acid cascade fits like a glove with the concept that inflammation involves the hyperactivation of a physiological process. For example, NSAIDs reduce mucosal hypersecretion in the common cold, along with a reduction in the physiological secretion that protects the gastric mucosa [19]. In fact, it has been reported that half of the patients treated with NSAIDs have gastric erosions and 10–30% have gastric ulcers [20]. Therefore, the problem of overlap between physiological and pathological aspects of inflammation has remained unresolved.

Incidentally, steroidal anti-inflammatory drugs are different. Rather than showing antidenaturant and antiprostaglandin effects, they inhibit the functions of leukocytes, which participate in both innate and acquired inflammation [21]. Their mechanism of action is reminiscent of the stress "attack" reaction to dangerous and life-threatening events [22, 23]. This reaction consists of a threefold adrenal discharge

involving sympathetic catecholamines, glucocorticosteroids, and mineralcorticostseroids [24, 25]. Leukocyte functions [including the immune response] are temporarily set aside, as they are useless in acute emergencies. In essence, adrenal discharge activates the somatic and mental processes needed to cope with emergencies. During World War II, the Nazis administered an adrenal cortex extract to fighter pilots that mimicked the corresponding biological process. Steroidal anti-inflammatory drugs belong to the above stress discharge, including hydrocortisone (cortisol), which has been found to explain the remission of jaundice-induced rheumatoid arthritis [26, 27]. We will return to this phenomenon shortly.

Returning to the protein-centered inflammation hypothesis, it has been revived in different terms. The idea was that protein denaturation was a process in its own right, suitable for separate medical treatment [28, 29]. Similarly, paracetamol is an anti-inflammatory drug that shows selective antinociceptive and antifebrile effects. Based on this idea, the aforementioned protein denaturation assay [13, 14] was used to select drugs, with the exception that they had to be free of the antiphlogistic and related side effects that others sought.

3. Pharmacotherapeutic implications

Bendazac, also known as bendazolic acid, is the first selective antidenaturant drug that has undergone thorough preclinical evaluation and medical use. The drug teaches something about the theoretical and practical value of this approach. First, the antidenaturant action of bendazac is against several agents, including extreme pH, heat, UV, and sunlight [10, 30–34]. It follows that bendazac is not a free radical scavenger. Rather, it binds to reactive protein sites, thus providing a protective barrier against free radicals. This binding does not result in a significant change in protein architecture and function. This suggests that the antidenaturant action depends on Van der Waals forces, which differ from covalent and ionic forces. Rather, they depend on the fluctuating polarizations of neighboring particles as a consequence of quantum dynamics.

Considering the pharmacological profile of bendazac, its most striking feature is its antinecrotic action [10]. Therefore, clinical studies have focused on dystrophic conditions [35]. More specifically, bendazac has been tested and shown to be active against inflammatory and allergic dermatoses: contact dermatitis, occupational dermatitis, seborrheic dermatitis, diaper and childhood dermatitis, constitutional eczema, erythema and localized itching, urticaria, drug allergies, and insect bites [36–42].

Thus, the preclinical and clinical experience gathered with bendazac supported the idea that protein denaturation is a process in its own right, suitable for targeted medical treatment. It is noteworthy that bendazac has been shown to be active against cataracts, which consist of physical opacification of the lens [43–46]. This suggests that a protein-denaturing agent could affect other medical conditions, such as kidney and gallstones, which are triggered by a protein-denaturing core.

Bindarit is another selective antidenaturant drug [47]. It shares a pharmacological profile with bendazac, but its study focused on adjuvant-induced arthritis in rats. It consists of the injection of Freund's adjuvant (a fine suspension of dead tubercle bacilli in liquid kerosene) into the plantar pad of rats. The adjuvant produces a primary inflammatory lesion at the injection site, followed, after about 10–15 days, by secondary lesions in areas of the body distant from the injection site [48–51]. The secondary lesions are accompanied by humoral changes consisting of denatured

GLIMBAL-like (globulin-like migrating proteins) proteins, which have been detected in patients with rheumatoid arthritis [52]. Returning to bindarit, it selectively inhibits its secondary lesions and related humoral changes, whereas hydrocortisone- and aspirin-like drugs inhibit both primary and secondary lesions [47]. Subsequently, bindarit was found to prolong survival and reduce renal damage in murine autoimmune disease [53, 54]. At this point, available information suggests that: (A) protein denaturation triggers the secondary immune-inflammatory process by exposing antigens common to both denatured and native proteins; and (B) an antidenaturing drug may show a protective action against autoimmune conditions in the absence of immunosuppressive effects. The resulting medical opportunities are exciting, but nevertheless, after 20 years bindarit still remains a matter of preclinical research and working hypotheses. Why?

The answer deserves a separate comment. It goes back to 1961, the year of thalidomide when images of phocomelic infants went around the world. This tragedy sent a warning signal about the risks of the hitherto uncontrolled use of synthetic drugs. Since then, they have been subject to strict toxicological controls, which have increased safety but burdened and delayed medical use. At the same time, there has been a resurgence of interest in drugs of natural origin, such as hormones, vitamins, and vaccines. They are not necessarily safer than synthetic ones, but they have a history of how to use them safely.

"It is important for us to identify nature's powerful, if accidental, antidotes," said Philip Hench on the occasion of receiving the Nobel Prize for the discovery of hydrocortisone. A small but exciting opportunity in the field of natural antidotes came with the experimental reevaluation of jaundice, the natural phenomenon that had paved the way for the treatment of rheumatoid arthritis with hydrocortisone a quarter century earlier [26, 27]. In a nutshell, it was discovered that jaundice involves an inhibition of protein denaturation, which, based on the above data and ideas, could participate in the remission of rheumatoid arthritis [55]. This phenomenon was partly attributable to the antidenaturant bilirubin and bile salts, but the effective doses and concentrations were hardly compatible with medical use. Hence, the search for more convenient compounds. Among the natural substances examined so far [56–60], candidates for medical use include fatty acids belonging to the composition of cell membranes [61]. Like bile salts and bendazac, they also prevent hemolysis of erythrocytes [62–64]. Hopefully, they will not have the same fate as bindarit.

In this story focusing on protein denaturation, tendinitis occupies a marginal but significant position. The tendon consists mainly of collagen (80%), the most abundant, ubiquitous, and versatile protein in mammals [65]. The type I collagen in the tendon gives it the strength and elasticity needed to connect muscle to bone, transmitting the mechanical forces of muscle contraction to the skeletal system. Its denaturation results in a loss of its elastoplastic function, which is irreversible. Experience with collagen deficiency disease suggests that tendinitis could also be addressed with dietary measures that promote collagen regeneration [66]. This is the message of the biological evidence, which is different but sometimes as strong as the experimental evidence.

4. Conclusions

This long and intriguing story ends up under the banner of biology. In modern science, it has been pushed aside by molecular biology, which focuses on single details that are difficult to trace back to their overall meaning. It is a kind of intellectual,

rather than visual, myopia. Like the cells in which they are produced, proteins have a biological cycle: they are born, mature, age (denature) and disintegrate, giving rise to waste. Slags are normally recycled to synthesize new proteins that replace aged ones or are excreted. The problem of slag mainly affects so-called perennial cells, including lens cells and neurons that last a long time without renewing themselves and their proteins [67, 68]. This is the case with cataracts, which are caused by scoriae that blur vision. Bendazac shows that an antidenaturing agent could influence this condition by reducing the extent of protein denaturation [30]. Slag is also involved in brain proteinopathy, which is related to beta-amyloid denaturation resulting in the formation of aggregates and toxic metabolites [69, 70]. The use of high doses of aspirin, an antidenaturant, has been reported to reduce the prevalence of Alzheimer's dementia [71]. Unfortunately, NSAIDs are burdened with unavoidable side effects. Bendazac and bindarit are potentially a step forward because of their selective antidenaturant action. This article presents some additional natural compounds, which belong to the composition and apparently to the physiological modulation of the protein life cycle.

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