Why does colchicine, which interferes with microtubule formation, improve the condition of patients with acute gouty arthritis?

Colchicine has been used medicinally for centuries, but only in recent decades have its antiinflammatory properties on nondividing cells (like neutrophils) been linked to interference with the cellular organization of labile fibrillar systems concerned with structure and movement (1); specifically, it prevents the polymerization of tubulin dimers to form microtubules. Neutrophils were obvious targets for effects of colchicine, being the major cellular components of synovial fluid during gouty attacks. They were subsequently found to contain colchicine-sensitive microtubules, and a variety of their motile functions seemed affected by colchicine (2). In addition to motile functions, colchicine has been reported to inhibit the elaboration by neutrophils of crystal-induced chemotactic factor, interleukin-1, and leukotriene B4 (3), agents that recruit and stimulate proinflammatory cells and all of which are produced in response to exposure to crystals.

Recent work on acute gouty arthritis (4, 5) has begun to distinguish what may be considered the "spark" from the "fuel" of this inflammatory engine. The fuel is monosodium urate (MSU) crystals, which are generally found within neutrophils during attacks; once these cells begin ingesting crystals, they can generate their own chemotactic factors to recruit more of themselves. The spark(s) that attract the initial wave of neutrophils, on the other hand, may be cytokines, either generated within the joint by the interaction of crystals with mononuclear phagocytes, or arriving in the joint from other areas of inflammation or trauma. This could explain why gout and pseudogout (the latter provoked by crystals of calcium pyrophosphate [CPPD]) are often grafted on to other febrile, debilitating illnesses. Colchicine, then, may "dampen" the neutrophil response while potential sparks dissipate (4).

Although colchicine is a general antiinflammatory agent, it is a weak one, except in acute gouty arthritis and a few other conditions, especially those involving infiltrations of neutrophils (2). Even in gout, the major role of colchicine is prophylactic. During acute attacks, colchicine is most effective when used early. These considerations imply that there is a critical, early, colchicine-sensitive, proinflammatory event in acute gout and other colchicine-responsive disorders, an event that later on becomes less important for the propagation and amplification of the inflammatory response. It is reasonable to suspect that the basis for colchicine's relative potency in certain types of inflammation may lie at the biochemical level of cellular signal transduction.

A major problem in the study of stimulus/response coupling is that a given stimulus may activate several cellular transduction systems, either directly through surface receptor activation, or indirectly through downstream activation/modulation of interrelated pathways. For example, activatable signal transduction mechanisms in neutrophils include cyclic nucleotide synthesis (cAMP, possibly cGMP), several modes of phospholipid hydrolysis (driven by different classes of phospholipases: C, D, and A_2), elevation of cytosolic Ca^{2+}, influx of extracellular Ca^{2+}, and tyrosine (TYR) phosphorylation (6–8). The soluble agonist FMLP clearly activates, to some extent, all of these mechanisms. Thus, it is not an easy matter to identify the primary target for an inhibitor of unknown mechanism.

In this issue of *The Journal,* in the latest of a notable series of papers regarding the activation of neutrophils by inflammatory microcrystals (MSU and CPPD), Roberge et al. (3; authors' references 13, 15, 16, and 18) show that a characteristic pattern of protein TYR phosphorylation induced by these crystals (and not by unopsonized zymosan or by soluble agonists) is selectively inhibited by colchicine and other microtubule inhibitors, but not by non–tubulin-binding analogues or by two unrelated nonsteroidal antiinflammatory agents. Whereas inhibitory effects of colchicine have previously been noted on several crystal-induced activation pathways in neutrophils, these were probably not agonist-specific effects (9, 10; Roberge et al. [3] references 15, 16, and 18). More selectively, colchicine was reported to block the elevation of cytosolic Ca^{2+} induced by crystals but not that induced by FMLP (Roberge et al. [3] reference 18). The work of the Roberge et al. (3) may now provide the first indication of a fundamental biochemical step directly and specifically targeted by colchicine.

Recently, Burt et al. (11), who independently reported crystal-induced TYR phosphorylation in neutrophils, showed that briefly preexposing the cells to an inhibitor of TYR kinase greatly inhibited multiple crystal-induced responses, including degranulation, superoxide production, and the aforementioned elevation of Ca^{2+}. This lends further credence to the significance of TYR phosphorylation in crystal-induced inflammation. However, one might ask whether the TYR-phosphorylated substrates (3) represent the proximal steps of signal transduction itself, or are instead participating further downstream as regulators/effectors of cell response. A notable difference between soluble agonists and crystals is the timing of the TYR phosphorylation events: maximal response to FMLP occurs at 1 min postexposure, whereas with crystals it occurs 5–45 min later. Thus, it seems that TYR phosphorylation might serve quite different roles for different classes of stimuli.

The currently reported colchicine-sensitive, crystal-induced pattern of TYR phosphorylation may represent an important part of the transduction pathway in the critical, early, colchicine-sensitive, proinflammatory event postulated above. There are, however, a few caveats. First, these studies were carried out in a protein-free medium, which makes for cleaner biochemistry in the study of signal transduction and cellular regulation, but does not reflect the "soup" that these cells actually live in. Future work might examine these effects using crystals opsonized with serum or synovial fluid. Second, regarding the specificity of the phosphorylation pattern produced by crystals, one would like to see the response to particulates likely to be encountered in vivo, such as live bacteria, as well as to complexes of antigen and antibody. Immune complexes are important, as they seem to be the raison d'etre for neutrophil infiltration in, for example, rheumatoid arthritis, which does

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not respond to colchicine. Finally, it would be of interest to assess the effects of crystals and colchicine upon TYR phosphorylation events in mononuclear phagocytes, as the responses of these cells may represent the sparks of the acute inflammatory response.

In summary, the work of Roberge et al. (3) is likely to be highly significant in elucidating the mechanisms of neutrophil activation by crystals, as well as in reinforcing an expanding view of TYR kinases as normal, active, and important elements in regulating the functions of nondividing, fully differentiated cells. Identification of the relevant TYR-phosphorylated proteins in neutrophils, and correlation of their phosphorylation state with certain cellular functions, will no doubt be the subject of future study. From a clinical perspective, if their work does prove to reveal the mechanism of colchicine's therapeutic selectivity, it may enable the more rational design of improvements in the prophylaxis and treatment of a number of inflammatory diseases.

The next interesting question is, how might microtubule disruption interfere with crystal-induced phosphorylation? The answer to that will have to be another chapter in the long and intriguing history of colchicine and gout.

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