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Dance Round

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

IS THERE A COMMON THERAPEUTICAL STRATEGY FOR BONE JOINTS AND BLOOD VESSELS?

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Growing evidence demonstrated recently a rationale for the use of orally administered collagen hydrolysate (collagen peptides) in the therapy of patients with osteoarthritis or other arthrodegenerative disorders. At the same time, there is a need for an effective treatment for millions of people in the world with atherosclerosis and its complications mainly due to the rupture of fibrous (collagenous-muscle) cap of the atherosclerotic plaque. Aortic aneurysm dissection in the heritable connective tissue disorders like Marfan, Ehlers-Danlos and Loeys-Dietz syndromes should also be considered herein. This Dance round article argues that the clinical data of collagen hydrolysate and matrix metalloproteinases (e.g., MMP-2, -9) inhibitors might be translated from osteoarthritis to the therapy of atherosclerosis as fibrous plaque stabilizers – this would be a joint of bone joints and blood vessels in action. ***Biomed Rev*** **2022; 33: 89-94**

Keywords: osteoarthritis, collagen hydrolysate, atherosclerosis, fibrous cap,vascular smooth muscle cells, protein secretion, matrix metalloproteinases, doxycycline

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INTRODUCTION

Osteoarthritis (OA), the most common form of arthritis, is a joint degenerative biomechanical disorder involving inflammation and thus resulting in cartilage and adjoining bone degradation. Orally administered collagen hydrolysate (collagen peptides) is absorbed intestinally and may accumulate in various organs, including cartilage (and fibrous cap of atherosclerotic plaque?) stimulating the production of collagen and proteoglycans and exerting anti-inflammatoy action**.** Hence, the oral intake of collagen hydrolysate has been proposed and is now being used as a nutraceutical to improve joint health in patients with OA.

Not only does the body make less collagen with age, but its quality is also not the same as in younger bodies. Collagen supplements have been investigated as a treatment for aging skin, wound healing, OA, rheumatoid arthritis, and osteoporosis.

Collagens

There are 28 collagen types, numbered with Roman numerals (I–XXVIII), in the human body. These constitute up to 30% of its total protein mass, thus representing the most abundant proteins in mammals. In the human genome, 44 collagen genes code for polypeptide chains that are combined in diverse ways to form collagen fibrils. A characteristic feature of all fibrillar collagens is the presence of a triple helix composed of polypeptides α-chains, each of which contains one or more regions featured by the repeating amino acid motif $(Gly\text{-}X\text{-}Y)_n$, with proline and 4-hydroxyproline amino acids often found at the X and Y positions, respectively. The triple helix motif can represent up to 96% of collagen type I, whereas less than 10% of collagen type XII. Of note, food-derived collagen peptides particularly those consisting of proline and hydroxyproline might be a novel nutraceutical challenge.

Boosting the secretory pathways of chondrocytes and vascular smooth muscle cells aiming at therapeutic benefit for osteoarthritis and atherosclerosis, respectively

According to George Palade's classical concept and Gunter Blobel's signal hypothesis, the protein secretory pathway comprises of several intracellular steps including synthesis, post-translational modifications, sorting, targeting, storage (in case of a regulated *versus* a constitutive secretion) and, finally, exocytosis (Figs. 1, 2).

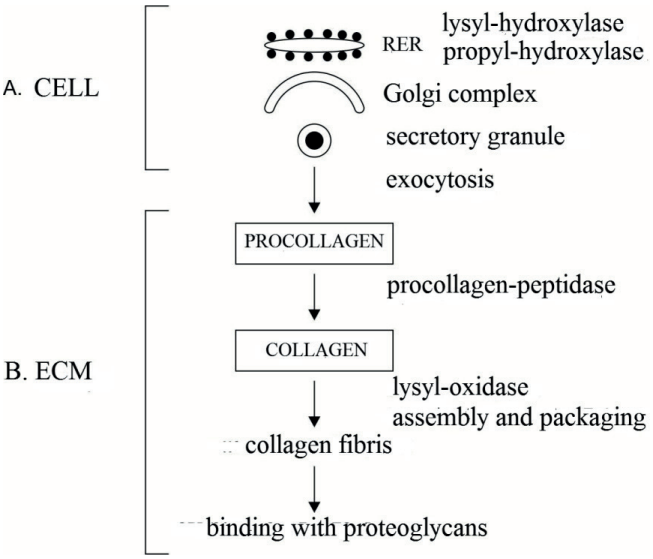

Figure 1. *A schematic illustration of intra- and extracellular secretory pathways of procollagen to collagen synthesis.* **From:** *Chaldakov GN. Principles of Cell Biology. 2021. BioMedES Ltd., Aberdeen, United Kingdom. Available in Amazon.com*

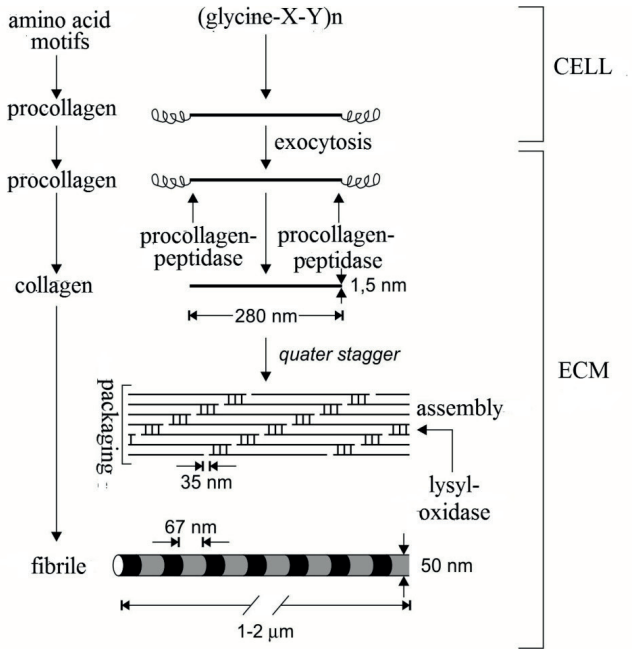

Figure 2*. A more detailed illustration of intra- and extracellular secretory pathways of procollagen to collagen biosynthesis and packaging*. **From:** *Chaldakov GN. Principles of Cell Biology. 2021. BioMedES Ltd., Aberdeen, United Kingdom.*

Accordingly, further studies on the pharmacology of matrix protein secretory pathways in both chondrocytes and vascular smooth muscle cells (SMC) are required, which would be based on the Palade-Blobel's general theory for protein secretion (see Kádár A, Ghenev PI, Tonchev AB, *et al* in this volume of *Biomedical Reviews*).

Lessons from collagen hydrolysate therapy for osteoarthritis

Collagen hydrolysate exerts a variety of biological activities including anti-inflammatory and anti-thrombotic alongside the stimulation of synthesis of collagen. Recent clinical investigations reported improvement of OA with nutraceuticals such as collagen hydrolysate and other collagen derivatives (1-4), for doxycycline, an inhibitor of MMP-2, -9, see (5).

Hence, the main focus of the present *Dance round* is whether these lessons could be translated into the clinical setting of atherosclerotic plaque stabilization.

FIBROUS CAP STRUCTURE AND FUNCTION

There is a general consensus that the majority of coronary syndromes result from the rupture of unstable plaques and associated thrombotic events. Plaque instability is strongly associated with disruption of the fibrous cap, an atheroprotective arterial layer. These data are interpreted as evidence that plaques which contain a high ratio of MMP/TIMP (matrix metalloproteinases/tissue inhibitors of metalloproteinases) and macrophages/SMC are prone to erosion and rupture.

Taken together, the results provide evidence that the balance of activity of MMP/TIMP and of SMC phenotypic modulation harbour the potential to moderate all processes of atherogenesis – from formation, development and progression to plaque stability-instability. The destruction of collagens, proteoglycans and other extracellular matrix proteins of the fibrous cap, due to elevated MMP and reduced TIMP activity, is considered to precipitate plaque rupture and its clinical sequelae such as myocardial infarction and stroke. In short, in stable plaques the lipid core is protected from the circulating blood by a fibrous (collagenous) cap. The thin fibrous cap contains many macrophages and lymphocytes, few SMC and less collagen fibrils, leaving the plaque prone to rupture and thrombosis (Table 1, Fig. 3).

Table 1. *Major features of unstable atherosclerotic plaque**

Thin fibrous cap (< 100 micrometer)
Increased presence of macrophages and lymphocytes
Low presence of SMC and collagen fibers
Large lipid core (> 30% of the total volume of plaque)
Presence of intra-plaque hemorrhage
Cap erosion and thrombus formation
Increased MMP/TIMP ratio
Increased expression of CD147, a MMP inducer
Altered expression of ADAM and ADAMTS
Increased expression of furin (PCSK3)
Increased expression of *transcription factor 21 (TCF21)* gene

***From:** Yanev S, Zhelyazkova-Savova M, Chaldakov GN. The fibrous cap: a promising target in the pharmacotherapy of atherosclerosis. *Biomed Rev* 2019; 30: 136-141.

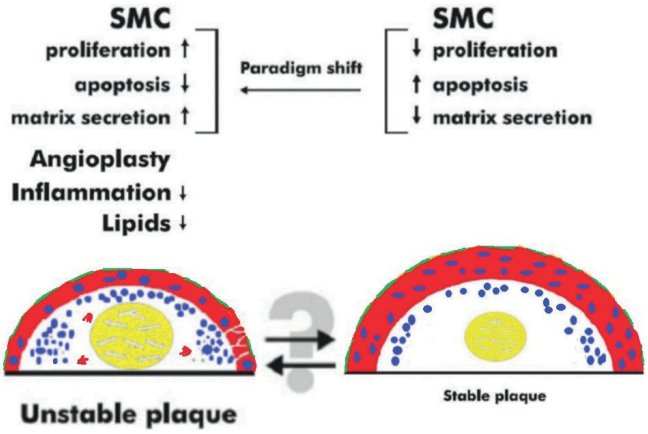

Figure 3. **Upper part,** *an illustration of the paradigm shift in the process of atherogenesis (long arrow), SMC, smooth muscle cells*. **Lower part,** *both plaques are covered by a single layer of endothelial cells (green) and fibrous caps (red). The unstable plaque has a thin fibrous cap containing macrophages (blue granules) and few SMC (blue spindles), which* may weaken the *cap, leaving it vulnerable to rupture (white lines at the right shoulder of the unstable plaque). To the contrary, the stable plaque has a thick fibrous cap composed of many SMC (blue spindles) and few macrophages (blue granules). Within the plaque, the lipid core (yellow) is larger in unstable than in stable plaques, and macrophages and lymphocytes (blue granules) are more prevalent in unstable than in stable plaques. In the unstable plaque, three intraplaque hemorrhages (red) are also depicted. In effect, a therapeutic strategy for promoting beneficial changes in MMP/TIMP ratio, also SMC phenotype, could be a viable avenue for addressing the stability of atherosclerotic plaque.*

FACTORS INVOLVED IN PLAQUE STABILIZATION

Possible major ways for plaque stabilization are: (i) making plaque's cap more fibrous, (ii) attenuating inflammation and oxidative stress, and (iii) reducing plaque lipid content. The first way represents the scope of the present *Dance round* article.

MAKING PLAQUE'S CAP MORE FIBROUS: METALLOPROTEINASES

The MMP family of proteinases consists of 26 zinc-dependent endopeptidases (containing zinc and methionine in its catalytic domain, hence, the name *metzincins*). They include MMP and **a** **d**isintegrin **a**nd **m**etalloproteinases (ADAM) and ADAM with **t**hrombo**s**pondin motifs (ADAMTS) and their endogenous regulators TIMP.

Mechanistically, since MMP may favor the degradation of matrix collagens and proteoglycans, the decrease of MMP activity with doxycycline, a non-selective MMP inhibitor, may have a stabilizing action on the fibrous cap (6–9). At the same stream, doxycycline was reported to improve aortic contractility and elastic fiber structure in a Marfan mouse aorta thus significantly delays thoracic aorta aneurysm rupture in Marfan syndrome-like mice (10, 11).

A list of plaque stabilizers is shown in Table 2.

Table 2. *A selective list of possible stabilizers of the atherosclerotic plaque*

- Collagen hydrolysate (collagen peptides)
- Inhibitors of MMP
  - ✓ Doxycycline
  - ✓ Macrolides (Clarithromycin, Erythromycin, Roxithromycin)
  - ✓ Angiotensin receptor blockers (e.g., losartan)
  - ✓ Furin (PCSK-3) inhibitors
  - ✓ CD147 inhibitors (Meplazumab)
  - ✓ Sodium butyrate*
- Activators of TIMP
- Modulators of ADAM and ADAMTS expression

*Sodium butyrate reduces the expression of proinflammatory mediators, also metalloproteinase production and limits the loss of type II collagen in IL-1β-inflamed chondrocytes. Thus, sodium butyrate may be a novel candidate in a multi-target approach for the treatment of chondrocyte inflammation and cartilage degenerative process in osteoarthritis (12).

MMP/TIMP ratio: biomarker and therapeutic target for atherosclerosis

Tissue inhibitors of MMP (TIMP-1, 2) play a key homeostatic role in regulating the activity of MMP and ADAM, and as such they are commonly increased when MMP activity is prevalent. Thus, the MMP/TIMP ratios might serve as biomarkers and therapeutic targets for atherosclerosis and other cardiovascular diseases such as Marfan syndrome and other hereditary connective tissue disorders. Circulating MMP-2 and MMP-9 (those that can be determined in the blood) may serve for the identification of patients who could profit from the drugs that inhibit MMP-2 and MMP-9 such as doxycycline (also see 13-41; for collagen hydrolysate in atherosclerosis, see 42-44).

CODA

The present *Dance round* describes an insight learned from the joint disease OA and associated disorders and relates them to the therapy of atherosclerosis and some hereditary connective tissue diseases like Marfan syndrome and related disorders. It also explores the potential therapeutic benefits of collagen hydrolysate and MMP inhibitors for atherosclerotic fibrous cap stabilization, hence the prevention of its rupture and, consequentially, the occurrence of myocardial infarction and ischemic stroke. We argue that search for selective MMP inhibitors and TIMP stimulators may be a novel promise for MMP-TIMP-dependent disorders we are *dancing round* herein. *But the secret sits in the middle and* does not know how exactly the orally taken collagen hydrolysate accumulates in diseased joints and vascular walls and how stimulates collagen and proteoglycan biosynthesis.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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