The Role of hyCURE® and Other Chondroprotective Agents in Oral Dietary Supplements

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Alternative therapies are predicted to be the modality of the new millennium. According to the Dietary Supplement Health and Education Act of 1994 (DSHEA), alternative therapy products are classified as "dietary supplements." In the DSHEA, a dietary supplement is defined as a product intended to supplement the diet and contains one or more of the following: amino acid, vitamin, mineral, or a herb or other botanical. It is the goal of this paper to provide an overview of the importance and synergies of *hy*CURE®, a protein comprising amino acids described as a hydrolysate of collagen, and other chondroprotective agents described herein as glycosaminoglycans (GAGs).

Chondroprotective agents are compounds such as *hy*CURE®[1] and other mucopolysaccharides (glycosaminoglycans)[2] that support or enhance the connective tissue matrix and, as such, are important structural components of cell membranes.[3] They are known to enhance synthesis of hyaluronic acid by synoviocytes and enhance chondrocyte (cells that rebuild damaged tissue) macromolecular synthesis.[4] It has been found that certain glycosaminoglycans, such as glucosamine sulfate or glucosamine hydrochloride and chondroitin sulfate, when given systemically or by injection provide reduced inflammation and stimulate mucopolysaccharide (glycosaminoglycan) synthesis. Synthesis occurs through extracellular as well as intracellular mechanisms.[5] Body linings and coverings are covered by endothelial, mesothelial or epithelial cells. Endothelial cells can be found on the inside of body organs such as blood vessels and in the cornea. Mesothelial cells are the flat squamous cells which cover the intestines and the true serous membranes of the body cavity including the peritoneum, the pleural cavity and the pericardium. Epithelial cells are primarily found covering the outside surfaces of the body, but also line the esophagus and the inside of the mouth.

Some of the most recent published literature presents observations on the structure-activity relationship of these agents. Glycosaminoglycans are reported to react with the "bricks" of the connective tissue, collagen and elastin, to maintain the normal structural and functional integrity of the joints, arteries, skin and other tissues.[6] These building blocks form cross-linkages with proteins such as collagen, the basis of cartilage. Proteoglycans are the major component of the amorphous ground substance of cartilage. Chondroitin sulfates are an integral part of this macromolecule, possessing an electric charge which favors an electrochemical attraction with water. The fluidity and compressibility of joint/tissue movement are

due to this arrangement of water containing molecules. Analysis of young tissue to old tissue reveals biological patterns that indicate young tissue is relatively uncross linked, maintaining high elasticity. Measurable differences of sulfonated chondroitin affecting the structure of the tissue may result in tissue stress.

The extracellular matrix is composed of various collagens, glycosaminoglycans, and elastin bathed by a tissue fluid found throughout the interstitial space.[7] It is the substratum in which fibroblast and macrophages normally reside, where fibroblast phenotypic transformation occurs, and into which inflammatory cells migrate when called upon during the process of tissue repair.[8] Spontaneous wound healing occurs partly by wound contraction, a process that requires intact functioning fibroblasts, and collagen production.[9]

There are many biological sources for glycosaminoglycans including porcine tissue, bovine tissue, shellfish and plants. Supply from shellfish is often complicated by erratic supply and contaminated raw sources. Many plant sources for glycosaminoglycans are available, but when taken orally, digestion of these by humans is more difficult. Glycosaminoglycans derived from animal sources are most frequently used; however, bovine tissues such as trachea and skin provide the most abundant source.

Glycosaminoglycans can be prepared from the principal solids of cartilage (connective tissue which helps to provide support and shape tissue). The principal solids of cartilage are chondromucoid (mucopolysaccharide), chrondroalbumoid and collagen. The chief component of cartilage was found by Miller in 1937 to be chondroitin sulfate(CS). Meyer in 1955 identified chondroitin sulfate as a repeating disaccharide (glucuronic acid and sulfated N-acetylalactosamine) and coined the term "mucopolysaccharide" to describe it.[10] Because mucopolysaccharides such as CS carry sugar molecules and acid groups (COO3) with each disaccharide unit, the CS structure favors an electrochemical attraction with water. This water loving property affects positively the structure and mobility of the connective tissue.

Chondroitin sulfates are produced by cells called chondrocytes, found primarily in cartilage and connective tissue.[11] As human beings age, the chondroitin sulfates that are produced by the chondrocytes decrease contributing to wrinkled skin, arthritis and other ailments. Supplemental chondroitin sulfates have been part of nutritional intake studies. These studies have shown that the consumption of chondroitin sulfate resulted in measurable boosts in their levels within the affected tissues.[12]

Studies show that chondroitin sulfates regulate the formation of new cartilage by stimulating chondrocyte metabolism and by the synthesis of collagen, proteoglycan and hyaluronic acid.[13] Additionally, chondroitin sulfates inhibit proteolytic and lysomolal enzymes (hyaluronidase) which may damage joint cartilage.[14]

Glucosamine is an aminomonosaccharide synthesized by the body. It is defined as one of the basic constituents of the disaccharide units of articular cartilage glycosaminoglycans (GAGs).[15] Glucosamine is a precursor and stimulatory agent for chondrocyte and connective tissue GAG synthesis.[16] Studies have shown that oral glucosamine is 98% absorbed.[17,18]

The hydrolysis of Type I collagen (*hy*CURE®) results in a spectrum of amino acids that differ greatly from that of other proteins by its high content of glycine and proline. These and 20 other amino acids, some of which are essential amino acids (cannot be manufactured by the body) and some are non-essential amino acids (can be manufactured by the body), are referred to as the "building blocks" of bones, muscles, and virtually all of the body's soft tissue, for repair and growth.[19] In addition to building cells and repairing tissue, they form antibodies to combat invading bacteria and viruses; they are part of the enzyme and hormonal system; they build nuclei proteins (RNA & DNA). When protein is broken down by the digestive system, the result is 22 known amino acids.[20]

*hy*CURE® collagen contains two unusual amino acids not found in other proteins, hydroxyproline and hydroxylysine.[21]

Hydroxyproline-rich collagen occurring at 12-14% of composition has been suggested as having an important role in cell-attachment.[22] The importance linked to hydroxylysine is its necessity for intermolecular collagen cross-linking and serving as an attachment site for carbohydrate.[23,24]

Table 1 illustrates a typical amino acid composition of a collagen hydrolysate.

Amino Acid	Typical Composition (%)
Glycine	20.0-30.5
Alanine	8.0-11.0
Serine	2.9-4.1
* Threonine	1.8-2.6
Proline	13.7-18.0
Hydroxyproline (only found in collagen)	12.1-14.5
* Valine	2.1-3.4
* Isoleucine	1.3-1.8
* Leucine	2.8-3.5
* Phenylalanine	1.1-2.6
Tyrosine	0.2-1.0
Cystine/cysteine	0.0-0.9
* Methionine	0.7-0.9
Aspartic acid	5.7-9.0
Glutamic acid	10.0-11.7

	Arginine	7.3-9.0
*	Histidine	0.7-1.0
*	Lysine	3.9-5.2
	Hydroxylysine (only found in collagen)	0.7-1.2

* essential amino acids

When taken orally, the chain of amino acids are broken up in the digestive tract by enzymes and acids. The individual amino acids are absorbed through the wall of the small intestine and into the bloodstream via the liver. From there, they travel to the sites that require formation and repair of tissue.

A synergistic rather than an additive effect is expected by combining glucosamine, chondroitin sulfate and *hy*CURE®, hydrolysated collagen. All contain properties that, whether extracellular or intercellular, are endogenous to cell repair and growth.

Referring to Table 1, a discussion of the remaining amino acids relative to this writing follows.

Glycine - non-essential. Helps trigger the release of oxygen to the energy requiring cell-making process. Important in the manufacturing of hormones responsible for a strong immune system.

Alanine - non-essential. Important source of energy for muscle tissue, the brain and central nervous system. Helps metabolize sugars and organic acids. Strengthens the immune system by producing antibodies.

Proline - non-essential. Extremely important for the proper functioning of joints and tendons.

Aspartic Acid - non-essential. Aids in the dispulsion of ammonia from the body.

Glutamic Acid - non-essential. Speeds the healing process and cell development.

Arginine - non-essential. Promotes cell growth; considered important for optimal muscle growth and tissue repair.

Leucine - essential. Provides ingredients for manufacture of other biochemical components in the body.

Lysine - essential. Helps form collagen and connective tissue.

DISCUSSION

The findings of previous reports seemingly are in concordance with the thrust of this writing. It has been found that severe malnutrition has adverse effects on wound healing in surgical patients. The value of nutritional support in the management of intestinal fistulas and inflammatory intestinal disease has been established by the studies of Dudrick[25], Vogel, et al[26], and Voitk, et al.[27] In a study conducted by Irvin[28], the effect of hyperalimentation on the healing of skin, abdominal and colonic wounds in malnourished rats was examined. In this study of 90 male Wistar rats, 70 rats were given a protein free diet, and ten of these rats also received an amino acid supplement. The rats that were protein free had a body weight reduction of 44%. These rats also had a reduction in the breaking strength of the skin and inflicted abdominal wounds. In the rats that were given the amino acid compound, significantly stronger abdominal wounds exemplified enhanced wound healing. The collagen content of these wounds was significantly greater than the collagen content of abdominal wounds in untreated rats.

Journal searches revealed a study by Lewis[29] in which he reports on 26 studies involving protein levels and the aetiology of pressure sores. The importance of nutrition is discussed specific to pressure sores requiring nutrients for healing, as with all wounds and obtained in diet. "Protein is essential for the formation of antibodies and leucocytes, fibroplasia, wound contraction and collagen synthesis."

It has been shown by Michaeli[30] that vastly improved wound healing is achieved by contacting the wound surfaces with collagen and a glycosaminoglycan. The healing process is brought about by complex biological mechanisms generally involving leucocytes and proteins. The application of collagen and GAG promotes the vascularization of the wound, attracts fibroblasts and endothelial cells by chemotaxis, and provides a favorable environment for the cells to participate in the healing process.

In a study reported by McCarty, it was found that the addition of glucosamine to culture-derived fibroblasts increased the secretion of mucopolysaccharides (GAGs) and collagen. Animal models have shown that oral glucosamine and chondroitin sulfate are incorporated in the articular cartilage.[32] These findings are in agreement with previous reports concerning the synergistic effects of GAG/CS in forming GAGs, inhibiting degradative enzymes, and enhancing cartilage metabolism and matrix production.[33,34]

Daily oral dosing used in clinical trials for glucosamine and chondroitin sulfates varied depending on the species and need to effectuate tissue protection and repair.[35,36,37] The author recommends an oral dosage be given according to patient

weight. For each kilogram of body weight, human or animal: 5 mg glucosamine, 3.5 mg chondroitin sulfate and 4 mg *hy*CURE®, hydrolyzed collagen.

Acute toxicity studies have found *hydrolyzed collagen* nontoxic when administered orally to mice and rats.[38,39] Dermal studies, when applied to rabbits and guinea pigs, gave no indication of systemic toxicity.[40,41] *Hydrolyzed collagen* was minimally irritating when evaluated for ocular irritation by a modified Draize eye test in rabbit eyes. In sensitization studies utilizing white guinea pigs, *hydrolyzed collagen* was found to be nonsensitizing. In phototoxicity/ photosensitization studies, hydrolyzed collagen was used to decrease UV-induced erythema.[42]

Glucosamine is almost completely absorbed, 98%, as demonstrated in human and animal studies and is nontoxic. Bucci[43] reports that oral dosages of 8 grams per kg. body weight to mice, rats, dogs and rabbits after months of dosing, as not causing any problems.

Safety testing conducted on chondroitin sulfate indicate that it is not a sensitizer, is non-cytotoxic and is not considered a primary dermal irritant.[44] Oral studies indicate that chondroitin sulfate is well tolerated and safe. McNamara concluded that there were clinically insignificant changes in hematological and hemostatic variables when tested in normal healthy dogs.[45]

*hy*CURE® collagen plays a pivotal role in the synthesis of glycosaminoglycans and other glycoproteins. Glycosaminoglycans and collagen are the chief structural elements of all connective tissues. Cartilage is composed of proteoglycans, collagen and the cells that rebuild damaged tissue, chondrocytes.[46] The pathogenesis of osteoarthritis involves the advancing loss of articular proteoglycan which results in an imbalance between synthesis and degradation.[31] Therapeutic amounts of glucosamine, chondroitin sulfate and *hy*CURE® collagen are recommended to stimulate the synthesis of collagen and glycosaminoglycans, thereby providing a natural tissue repair for arthritic conditions as well as connective tissue damage.

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