Experimental Diabetes as a Model of Accelerated Aging: Matrix Collagen Modifications

Hızlandırılmış Yaşlanma Modeli Olarak Deneysel Diyabet: Matriks Kollagen Modifikasyonları

Milan STEFEK,[a]
Çimen KARASU[b]

[a]Institute of Experimental Pharmacology and Toxicology,
Slovak Academy of Sciences,
Bratislava, SLOVAKIA
[b]Department of Medical Pharmacology,
Faculty of Medicine, Gazi University,
Ankara, TURKEY

Yazışma Adresi/*Correspondence:*
Milan STEFEK
Institute of Experimental Pharmacology and Toxicology,
Slovak Academy of Sciences,
Bratislava, SLOVAKIA
exfastfk@savba.sk

ABSTRACT Collagen, the main protein of the extracellular matrix, undergoes continual cross-linking during aging. In healthy individuals, this process is mediated by lysyl oxidase and proceeds slowly. In addition to physiological maturation, aging collagens are crosslinked by AGEs (advanced glycation end-products), formed by a reaction between reducing sugars and body proteins in a process of non-enzymatic glycoxidation. These pathological processes are accelerated in diabetic individuals, whose average blood glucose is higher than normal. In our studies, rat tail tendon mechanical strength was significantly enhanced for diabetic animals when compared with those of age-matched controls. Tail tendons from diabetic rats were found to contain elevated amounts of p-dimethylaminobenzaldehyde-reactive material with an absorbance spectrum characteristic of the Ehrlich chromogen. The characteristic glyco-fluorophore was elevated in tendon collagen of diabetic animals. The glycation inhibitor aminoguanidine significantly inhibited changes of all three parameters evaluated while the pyridoindole antioxidant stobadine significantly decreased only tendon mechanical strength.

Key Words: Collagen, Aging, Diabetes, Glycation, Antioxidant

ÖZET Ekstraselüler matriksin ana proteini olan kollagen yaşlanma sürecinde devamlı çapraz bağlanma geçirmektedir. Sağlıklı kişilerde daha yavaş ilerleyen bu sürece lizil oksidaz eşlik etmektedir. Fizyolojik tekamüle ilave olarak, yaşlanmış kollagenler, indirgenmiş şekerler ve proteinler arasında enzimatik olmayan gliko-oksidasyon reaksiyonları ile şekillenen ileri glikasyon son ürünleri (AGEs) tarafından çarpaz bağlanmaktadırlar. Bu patolojik süreçler diyabetli kişilerde hiperglisemi sebebiyle hızlanmaktadır. Bizim çalışmalarımızda, sıçan kuyruk tendonunun mekanik direnme gücü diyabetik hayvanlarda kontrollere oranla anlamlı olarak artmıştır. Diyabetik sıçanların kuyruk tendonlarında p-dimetilaminobenzaldehit reaktif materyalinin miktarının, Elrich kromojeninin absorbans spectrum karakteristikleri ile, arttığı bulunmuştur. Karakteristik gliko-florofor, diyabetik hayvanların tendon kollejenlerinde yükselmiştir. Pridoindol antioksidan stobadin sıçan kuyruk tendonunun sadece mekanik direnme gücünü azaltırken, glikasyon inhibitörü aminoguanidin, her üç parametrede saptanan değişiklikleri anlamlı olarak inhibe ettiği gösterilmiştir.

Anahtar Kelimeler: Kollagen, Yaşlanma, Diyabet, Glikasyon, Antioksidan

Collagen is the most abundant protein in the body making up about 25% to 35% of the whole-body protein content. Collagen fibers are a major component of the extracellular matrix that supports most tissues including fascia, cartilage, ligaments, tendons, bone and skin. Along with soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to wrinkles that ac-

company aging. It strengthens blood vessels and plays a role in tissue development. Age-related cross-linking of collagen results in joint, myocardial and vascular stiffness with corresponding pathological consequences.

The fundamental questions related to biochemistry of collagen aging are: 1) What are the molecular mechanisms responsible for the age-related changes of matrix collagen ? 2) Is there any chance to slow down these processes ?

MATRIX COLLAGEN MODIFICATIONS IN AGING AND DIABETES

An excursion to the history of aging biology would lead us to Fritz Versár who is considered a founder of experimental gerontology. His fundamental experiments, performed in the 1950s on aging of rat tail tendons, represented the first demonstration of age-dependent modifications of the extracellular matrix: an exponential increase of mechanical strength of collagen fibers with age (Versár´s phenomenon).[1]

At the time of Versár´s experiments, molecular mechanisms of the structural changes in aging collagen were not known. These questions were not resolved until the 1980s, when Monnier and Cerami came with the glycation theory of aging.[2] According to this hypothesis, glucose is the key mediator of aging. Glucose as a polyhydroxy aldehyde binds to a free amino group of a protein to yield a Schiff base. Schiff base, rather unstable aldimine, changes to a more stable ketoamine, the Amadori product, which accumulates in tissues. Yet, the Amadori product is not the final result of the glycation reaction since it slowly undergoes a series of mostly ill-defined transformations to yield a rather heterogeneous group of final stable products, advanced glycation endproducts (AGEs). The processes of advanced glycation were very soon found to be tightly interconnected with oxidative modifications since, in addition to reactive oxygen species generated in various metabolic pathways, glucose itself, free or bound into the Amadori product, can contribute to free radical generation via auto-oxidation reactions. The final complex process of the structural changes initiated by glucose was termed glycoxidation.[3] These processes have some overlap with lipid peroxidation since the reactive aldehydes, products of lipid oxidation, may contribute to crosslinking of proteins. As a matter of fact, the term glycoxidation reflects natural joining of two apparently independent theories of aging : the free radical theory introduced by Harman[4] in the 1950s and the glycation theory.

Collagens, the main proteins of the extracellular matrix, undergo continual cross-linking during aging. In healthy individuals, this process is mediated by lysyl oxidase and proceeds slowly as the body ages, apparently leveling off in early adulthood. Evolution has tailored this process to produce collagen fibrils with an optimal balance of strength and flexibility.[5] As shown above, in addition to physiological maturation, aging collagens are crosslinked by formation of AGEs. Collagens are especially exposed to glycation because they contain several lysine, hydroxylysine and arginine residues with free amino groups. Further, they have a slow turn over rate and are exposed to ambient level of glucose. AGE-mediated cross-linking is a true aging process with multiple deleterious functional consequences including increased tissue stiffness, resistance to enzyme degradation, pathological surface changes, overactivation of tissue repair mechanisms, etc.[6]

It is significant that these pathological processes are accelerated in diabetic individuals, whose average blood glucose is higher than normal. Indeed in diabetic patients severe health complications may evolve in younger age: they include ischemic heart disease and stroke, retinopathy and cataract, renal disease, neuropathy, lower limb amputations, etc. As a result of these deadly health complications, mortality of poorly controlled diabetics may significantly increase compared with the general population. There is a great deal of evidence showing that, at molecular level, processes of non-enzymatic glycation/glycoxidation of the endogenous proteins, including collagen, contribute to the development of these health disorders.[7]

Using a model of streptozotocin-induced diabetes in rats, the time-dependent changes of tail tendon mechanical properties along with alterations in markers of tendon collagen glyco-oxidation were evaluated. The effects of the glycation inhibitor aminoguanidine and the pyridoindole antioxidant stobadine were assessed.

Tendon breaking time, determined as a measure of collagen cross-linking, was significantly increased in diabetic rats (Fig. 1a). Pepsin digests of tail tendons from diabetic rats were found to accumulate material that reacted rapidly with p-dimethylaminobenzaldehyde to give an adduct with an absorbance maximum at about 572 nm (Fig. 1b), characteristic for the Ehrlich chromogen of pyrrolic nature determined in ageing collagens8. Moreover, collagen obtained from tail tendons of diabetic animals showed increased fluorescence (Fig. 1c) with excitation and emission maxima of 365 nm and 416 nm,
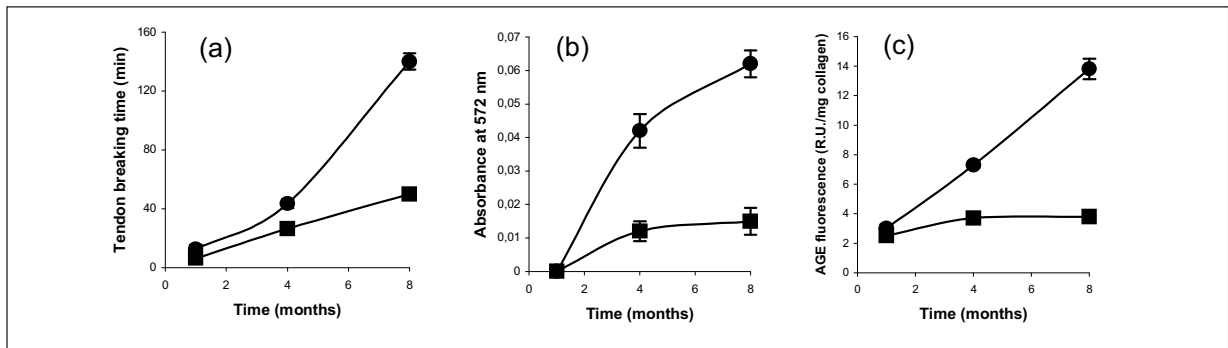
FIGURE 1: Diabetes-induced changes of rat tail tendon collagen. Time dependence. Tendon breaking time , (b) Ehrlich adduct absorbance at 572 nm, (c) AGE-related fluorescence (excitation, 365 nm; emission, 416 nm). (■) - control animals (n=8), (●) – untreated diabetic animals (n=17). Results are mean values ± SEM9.

respectively, characteristic of products of advanced glycation.[8,9]

As shown in Figs. 2a and 2b , both the tendon breaking time values and AGE-related fluorescence of tendon collagen correlated positively with the Ehrlich adduct absorbance underlining the cross-linking and AGE-related nature of the Ehrlich chromogen moiety. Indeed, non-enzymatic processes of advanced glycation may be implicitly involved in the formation of pyrrole structures in collagen, e.g. via chemical reaction of sugar-derived intermediary alpha-dicarbonyls with protein amino acid residues.[10]

The glycation inhibitor aminoguanidine significantly inhibited changes of all three parameters evaluated[8,9]. Treatment of diabetic animals with stobadine partially normalized tendon mechanical strength, while the glycation-related fluorescence and Ehrlich chromogen absorbance remained unaffected (Fig. 3).[9,11] The inhibitory effect of aminoguanidine supports the participation of mechanisms related to non-enzymatic advanced glycation. The discordant effects of stobadine

on mechanical properties of tail tendons on one hand and AGE-related fluorescence and Ehrlich chromogen absorbance on the other, suggest that additional mechanisms different from advanced glycation may also participate in collagen cross-linking in diabetic connective tissues; hyperglycemia-induced oxidative processes may be the most likely candidates.

Accelerated advanced glycation processes in diabetic rats, as detected by Ehrlich's positive material and collagen linked fluorescence in the tail tendons, were reduced significantly by the green tea extract.[12]

CONCLUSION

Biochemical changes of matrix collagen in experimentally diabetic rats support the concept of glucose-induced damage in diabetes via tightly interconnected glycation and oxidation (glyco-oxidation) mechanisms. Thus, besides the classical treatments of diabetes, strategies involving antioxidants and anti-glycation agents may represent a promising means of adjunct therapy to prevent the progression of diabetic complications.
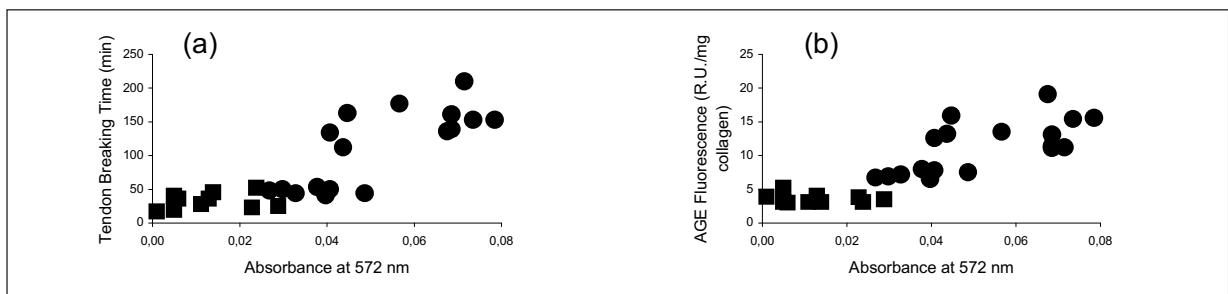
FIGURE 2: Diabetes-induced changes of rat tail tendon collagen in relation to Ehrlich adduct absorbance. (a) Tendon breaking time vs. Ehrlich adduct absorbance (r = 0.84) , (b) AGE-related fluorescence (excitation, 365 nm; emission, 416 nm) vs. Ehrlich adduct absorbance (r = 0.84). The results were obtained in the 4th and 8th month; (■) - control animals (group C),   (●) - untreated diabetic animals (group D);   p < 0.001, diabetic animals vs. age-matched controls for each parameter meas-
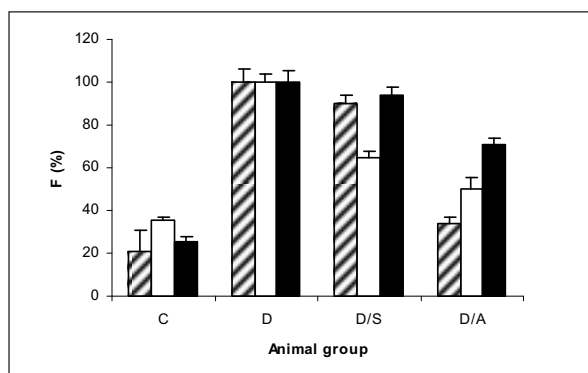
FIGURE 3: Changes of tail tendon collagen in streptozotocin-diabetic rats. Effect of aminoguanidine and stobadine. F - values represent Ehrlich adduct absorbance (hatched bars), tendon breaking time (white bars) and AGE-related fluorescence (black bars) expressed as percentage of the respective values obtained for untreated diabetic animals (group D). C - control rats, D/A – diabetic rats treated with aminoguanidine. D/S – diabetic rats treated with stobadine. Results were obtained at the end of the 8-month experiment and are expressed as mean values ± SEM (n = 8 – 17) [9,11]

On balance, if glycoxidation is accepted as a common element of aging and diabetic complications then: i) Diabetes can be considered as a model of premature aging; ii) Diabetes research is helping to investigate approaches to slowing down aging. The knowledge of mechanisms of aging at molecular level is of key importance to interfering efficiently with aging. If the glycoxidation theory is accepted as relevant for both the aging process and the development of diabetic complications, then glycation inhibitors and antioxidants developed by diabetes research to prevent and cure diabetic complications are expected to slow down aging mechanisms.

Acknowledgement

▮ REFERENCES

1   Robert L. An original approach to aging: an appreciation of Fritz Verzár's contribution in the light of the last 50 years of gerontological facts and thinking. Gerontology 2006; 52 (5): 268-74.

2   Monnier VM, Cerami A. Nonenzymatic browning in vivo: possible process for aging of long-lived proteins. Science 1981; 211: 491–493.

3   Baynes JW. Role of oxidative stress in development of complications in diabetes. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40 (4): 405-12. Review.

4   Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11 (3): 298-300.

5   Robins SP. Biochemistry and functional significance of collagen cross-linking. Biochem Soc Trans 2007; 35 (Pt 5): 849-52. Review.

6   Ulrich P, Cerami A. Protein glycation, diabetes, and aging. Recent Prog Horm Res 2001; 56: 1-21. Review.

7   Ahmed N. Advanced glycation endproducts--role in pathology of diabetic complications. Diabetes Res Clin Pract 2005; 67 (1): 3-21. Review.

8   Stefek M, Gajdosik A, Gajdosikova A, Krizanova L. p-Dimethylaminobenzaldehyde-reactive substances in tail tendon collagen of streptozotocin- diabetic rats: temporal relation to biomechanical properties and advanced glycation endproduct (AGE)-related fluorescence. Biochim Biophys Acta - Mol Basis Dis 2000; 1502 (3): 398-404.

9   Stefek M, Gajdosikova A, Gajdosik A, Kyselova Z, Djoubissie PO, Krizanova L. Glyco-oxidative mechanisms in glucose toxicity:biochemical changes of matrix collagen in diabetic rats. Biologia (Bratisl) 2005; 60

(Suppl.17): 109-112.

10   Deyl Z, Miksik I. Post-translational non-enzymatic modification of proteins. I. Chromatography of marker adducts with special emphasis to glycation reactions. J Chromatogr B Biomed Sci Appl 1997; 699 (1-2): 287-309. Review.

11   Stefek M, Gajdosik A, Tribulova N, Navarova J, Volkovova K, Weismann P, Gajdosikova A, Drimal J, Mihalova D. The pyridoindole antioxidant stobadine attenuates albuminuria, enzymuria, kidney lipid peroxidation and matrix collagen cross-linking in streptozotocin-induced diabetic rats. Methods Find Exp Clin Pharmacol 2002; 24 (9): 565-571.

12   Babu PV, Sabitha KE, Shyamaladevi CS. Effect of green tea extract on advanced glycation and cross-linking of tail tendon collagen in streptozotocin induced diabetic rats. Food Chem Toxicol 2008; 46 (1): 280-5.