Review

Assessment of the Substance Antioxidative Profile by Hyaluronan, Cu(II) and Ascorbate

Katarína Valachová * and Ladislav Šoltés

Centre of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská Cesta 9, SK-84104 Bratislava, Slovakia; ladislav.soltes@savba.sk
* Correspondence: katarina.valachova@savba.sk; Tel.: +421-232295707

Abstract: In the minireview presented here, the authors discuss the evaluation of inhibitory effect of substances in the phases of initiation and propagation of high-molar-mass hyaluronan oxidative degradation. The experimental approach should be considered as original since on using a simple experimental assay it is possible to prove both the so-called "preventive" and "chain-breaking" antioxidant activity of investigated water-soluble endo- or exogenous substances.

Keywords: preventive antioxidants; chain-breaking antioxidants; reactive oxygen species

Citation: Valachová, K.; Šoltés, L. Assessment of the Substance Antioxidative Profile by Hyaluronan, Cu(II) and Ascorbate. *Pharmaceutics* **2021**, *13*, 1815. https://doi.org/10.3390/pharmaceutics13111815

Academic Editor: Pietro Matricardi

Received: 7 October 2021
Accepted: 26 October 2021
Published: 31 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introductory Remarks

The website "Free Radical School Presentations" [1] serves as "an archive" for many of the Free Radical School Workshop and Virtual School lectures that were organized by the Society for Redox Biology and Medicine and presented at SfRBM's Annual Meeting within 15 years (the first one in 1997 and the last one in 2011). This website is intended to be a source for basic education and reference material for a wide variety of important topics in the field of redox research including the experimental methods to assess the substance antioxidative profile.

In the 1990s, the research activities of many scientific teams directly or indirectly focused on the thesis that antioxidants are the "miracle substances" that can effectively intervene in both the acute and chronic phases of many diseases, including aging. Reaction systems for evaluating the antioxidant activity of a substance also contain a generator of oxidant(s) and a probe whose intactness during the reaction proves the antioxidant activity of the substance assessed.

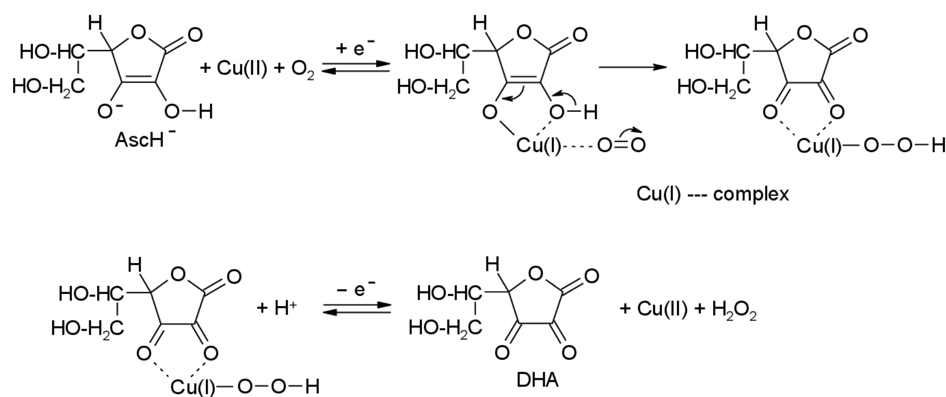
In this minireview the primary/initiating oxidants are the OH radicals, the secondary oxidants are alkyloxy- and alkylperoxy- type radicals. The probe is the organism's own macromolecule, namely a high-molar-mass hyaluronan. The kinetics of the hyaluronan oxidative degradation has been most properly monitored by changing the dynamic viscosity of the reaction solution.

2. Sequestration of Copper Cations with Ascorbate

The biogenic transition metal—copper—is found in the healthy human body as cupric and cuprous cations, whereas 95% of the total copper content is bound to the blood plasma protein—ceruloplasmin [2–4]. Each ceruloplasmin macromolecule binds eight copper cations, of which two are readily released. The normal level of serum ceruloplasmin concentration is in a range of 1.52–2.65 μmol/L. Thus, under physiological conditions the concentration of loosely bound copper (cupric/cuprous cations) can reach up to ≈0.66 μmol/L. The patho-physiological range of ceruloplasmin blood serum level is significantly increased, since this metalloprotein belongs to the acute phase reactants, the level of which rises during acute and chronic inflammations, infection, trauma, Alzheimer's disease, etc. Ascorbic acid/vitamin C, in the form of ascorbate, is ubiquitous in the human

body. The ascorbate molecule (Asc^-) in a living organism functions also as a coordinating low molar mass ligand. In case of ascorbate-iron pair, ascorbate may serve both as a Fe(III)/Fe(II) chelating agent and reductant of Fe(III) to Fe(II) cations. In latter case, under the aerobic conditions ascorbate with ferrous cation forms a coordination complex: ascorbate—Fe(II)—dioxygen [5]. This, the so called Udenfriend oxidative complex/system, is a very efficient oxidative agent used, e.g., by organic chemists to hydroxylate aromatic compounds, saturate hydrocarbons to alcohols, olefins to epoxides, etc. [6].

The paramagnetic bivalent Cu(II) (outermost orbitals = $3d^9$) represents the most stable oxidation state of copper. Since ascorbate acts as a powerful reducing agent with a standard reduction potential E'° of +0.282 V for the redox couple $Asc^{\bullet -}/Asc^-$ at pH 7, it should reduce Cu(II) to Cu(I). Thus, taking into account that the standard reduction potential of the pair Cu(II)/Cu(I) is +0.16 V, Cu(I) should be able to reduce O_2 molecules to yield directly H_2O_2. However, as often claimed, the so called Weissberger system—ascorbate—Cu(II/Cu(I)—dioxygen [7–9]—generates hydrogen peroxide molecules (cf. Scheme 1) [10–13], or due to the decomposition of H_2O_2 by Cu(I) complex through Fenton type reaction, the Weissberger system became one of the most potent generators of hydroxyl radicals [14–16].



Scheme 1. Generation of H_2O_2 via the Weissberger system composed of ascorbate and Cu(II) under aerobic conditions. $AscH^-$ represents ascorbate anion and DHA represents dehydroascorbate.

When the Weissberger system has no additional oxidizable substrate, we speak about ascorbate autoxidation [17]. In any case, it should be pointed out that the biological consequences of interactions of vitamin C with biogenic transition metal cations of iron, copper, or manganese have not been fully understood yet. Over the past decade, the pro-oxidant properties of ascorbate have been investigated in addition to its better explored antioxidant role.

When oxidizable substrates are simultaneously present, the reaction products consist of both oxidized/decomposed substrate [18] and DHA—dehydroascorbate, whose molecules in aqueous milieus hydrolyze fast to 2,3-diketo-L-gulonic acid [17]. Although for the next sections of this minireview the complexation of ascorbate with another biogenic transition metal is not essential, the reader could find some complexation kinetics and/or equilibrium data between ascorbate and several metal cations, e.g., in the paper by Fornaro and Coichev [19]. One of the frequently cited statements in literature is that copper cations released from the ceruloplasmin macromolecule are extensively entrapped by albumin present in the bloodstream. This tenet is naturally in part true, but it must also be admitted that the anions of ascorbate are the most probably co-responsible for the release of copper cations from ceruloplasmin. The charge–charge interaction between $AscH^-$ and, e.g., $[CuCl]^+$ with a rate constant of 280 $mol^{-1}\ L^{-1}\ s^{-1}$ [19] may outweigh the rate of gradual dissociation of the complex between $[AscH]^-$ and $[CuCl]^+$ followed by a subsequent association of Cu(II) with a copper fixing/binding site on the albumin molecule.)

3. Hyaluronan—Oxidizable Biological Substrate

Hyaluronic acid (hyaluronan; HA; Figure 1), or its salts, is a linear high-molar-mass natural polysaccharide formed from disaccharide units of regularly alternating *N*-acetyl-D-glucosamine (GlcNAc) and D-glucuronic acid (GlcA) units linked by β-(1→3) and β-(1→4) linkages. The chemical structure of HA is regular, the only exception is a possible replacement of *N*-acetyl-D-glucosamine by deacetylated glucosamine residues.

Figure 1. Hyaluronic acid.

A human body weighing about 70 kg contains approx. 15 g of HA [20], whereas one third of this amount is turned over every day. Such an unusually extensive de novo synthesis of HA megadalton macromolecules suggests that the functions of both native hyaluronan and its lower sized fragments in the body will be diverse [21]: In the body of vertebrates HA is abundantly present in almost all body fluids and tissues. In a fibrous tissue a capsule called synovium, one of the main components is the synovial fluid (SF), functions as a lubricant [22–28]. The SF in a healthy human being, along with the blood plasma filtrate, also contains the entangled macromolecules of HA {1.4–3.6 mg/mL [26]}. While in SF and vitreous humor HA macromolecules are not associated with proteins, the HA chains, filling the space between the collagen fibrils, provide elastic properties of these soft tissues [29–31]. Yet, HA in extracellular matrices is linked with several proteoglycans creating a scaffold in the forms of more or less hard tissues such as skin, umbilical cord, and cartilage [32–36].

Two ways of HA decay must be denoted here: first, enzymatic depolymerization, by which HA fragments of lower molar mass are formed, and second, oxidative degradation, a process which is of great interest to polymer chemists, biochemists and molecular biologists [37–41].

The half-life of HA depolymerization (by hyaluronidases) is in the range of 1–3 weeks in cartilage, 1–2 days in skin and only 2–5 min in blood plasma [42,43]. In contrast, in SF of healthy individuals, whose fluid lacks any hyaluronidases, the half-life of HA $\approx$ 12 h is suggestive of other than enzymatic decay [44]. Such a rapid turnover of HA in SF could be elucidated by a mechanical pumping-out of a part of SF through the lymphatic system during the day-time moving activity of a person. Another proposed mechanism is an oxidative degradation of several megadalton-HAs in SF of healthy individuals [45–48]. This tenet is supported indirectly by the fact that under physiological conditions, the concentration of ascorbate in SF of healthy humans reaches the values closely to those in blood plasma, i.e., 40–140 μmol/L [49,50].

Figure 2 illustrates the action of oxidative system comprising complex of ascorbate—Cu(II/Cu(I)—dioxygen: As evident, a megadalton-HA sample progressively degrades to intermediate-sized polymer fragments, which mean molar mass is reduced by one or even two orders of magnitude.

3.1. Hyaluronan Oxidative Degradation by Free-Radical-Chain Reaction

3.1.1. Initiation Reaction(s)

As shown in the sub-section "Sequestration of copper cations with ascorbate" for the generation of $^{\bullet}$OH radical(s) the so called Weissberger biogenic oxidative system can produce a continual flux of hydroxyl radicals [15,16]. (To simplify some reaction sequences appearing in this minireview, HA abbreviates the hyaluronan macromolecule. The $A^{\bullet}$ denotes a *C*-centered hyaluronan macroradical, a peroxy-type macroradical $AOO^{\bullet}$ along with a highly unstable alkoxy-type macroradical $AO^{\bullet}$ represents *O*-centered intermediate macroradicals).

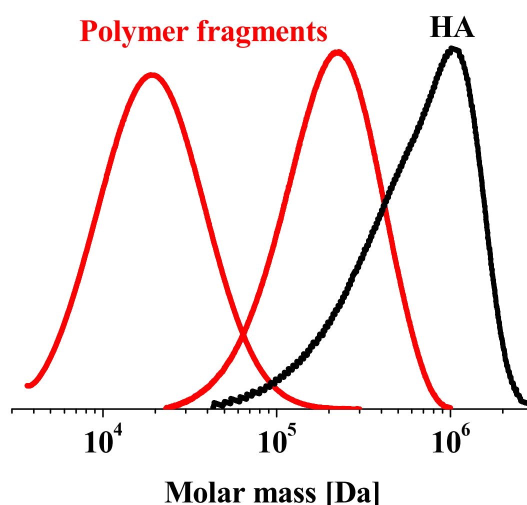
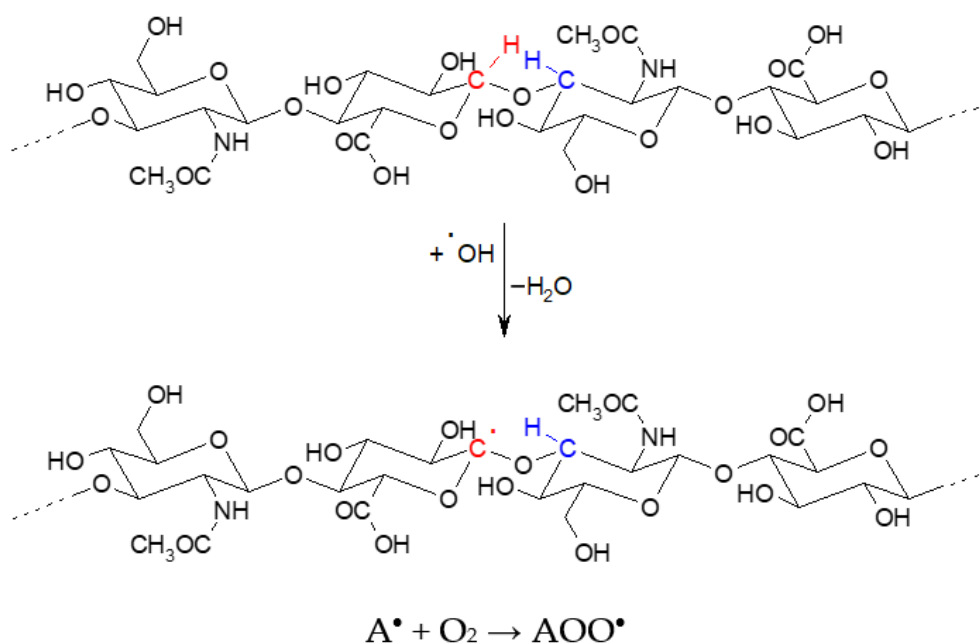


Figure 2. Comparison of the molar mass distribution of the megadalton HA sample and those ones degraded in situ under aerobic condition by action of $AscH^-$ and copper ions. Adapted from [51], published by Elsevier, 2007.

The following Scheme 2 describes structural chemical formulae the phase of initiation reaction(s) of high-molar-mass HA oxidative degradation:



$$A^{\bullet} + O_2 \rightarrow AOO^{\bullet}$$

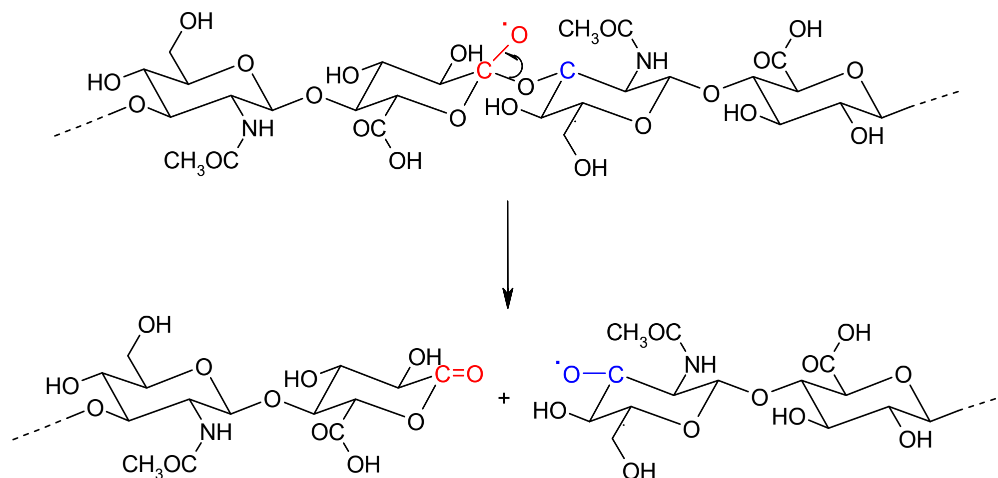
Scheme 2. An intact HA macromolecule (upper panel) reacts with $^{\bullet}OH$ radical yielding an intermediate $A^{\bullet}$, i.e., a *C*-centered hyaluronan macroradical (lower panel), which immediately reacts with a molecule of oxygen resulting in a peroxy-type macroradical $AOO^{\bullet}$.

The macroradical $AOO^{\bullet}$ can undergo the reaction with Cu(I) complex (cf. Scheme 1) yielding a highly unstable alkoxy-type macroradical. It is comprehensible that either $AOO^{\bullet}$ macroradical or that of $AO^{\bullet}$, along with the parent $A^{\bullet}$ intermediate macroradical, form a collection of highly reactive compounds, i.e., the initiators of subsequent self-perpetuating free-radical degradation of high-molar-mass HA.

3.1.2. Transfer of the Free-Radical Centre and Fragmentation Reaction(s)

As is well-known in macromolecular chemistry, a long chain alkoxy-type macroradical freely undergoes the strand scission due to the β-cleavage. Thus the scission at, e.g., C(1) on

the ring of D-glucuronic acid, yields polymer fragments, namely a macromolecule bearing a terminal C=O group and a novel alkoxy-type macroradical (cf. Scheme 3). As a rule of such a degradation reaction both fragments have reduced molar mass.



Scheme 3. The long chain alkoxy-type macroradical AO$^•$ undergoes a transfer of one electron at, e.g., C(1) on the ring of D-glucuronic acid (upper panel). The decay of the native chain alkoxy-type macroradical AO$^•$ yields two polymer fragments (lower panel) having reduced molar masses. The newly formed alkoxy-type macroradical of shorter molar size (AO$^•$) naturally could act as the initiator of the self-perpetuating free-radical HA degradation.

The initiating $^•$OH radical(s) (cf. Scheme 1) can react with the D-glucuronate/D-glucuronic acid and *N*-acetyl-D-glucosamine functional moieties by opening the alkyl rings [52–55] without breaking the HA chain.

3.1.3. Termination Reaction(s)

The free-radical degradation reactions of the native high-molar-mass hyaluronan belong to self-perpetuating reactions. One of the most effective procedures terminating the self-perpetuating reactions is to scavenge this sequence in the very phase of their initiation. Reactions represented in Scheme 1, subsequently followed by H_2O_2 decomposition, can be terminated by a so-called preventive antioxidant, e.g., a substance freely donating an atom of hydrogen, i.e., $^•$H: In such a case the substance, classifiable as HAT—hydrogen atom transferring, terminates the action of the $^•$OH radical and thus it interrupts the generation of A$^•$ macroradical. (It should be claimed here that at pH 7.4, more than 99.9% of ascorbic acid ($AscH_2$) is present dissociated as $AscH^-$. Thus, the antioxidant chemistry of vitamin C is the chemistry of ascorbate, which freely donates a hydrogen atom to an oxidizing radical).

According to the cascade of reactions showed in Schemes 2 and 3 it is obvious, that to prevent the propagation phase of the free-radical HA degradation one must apply an antioxidant which acts as HAT. Such a property is attributed to the so-called chain-breaking antioxidants. It is comprehensible that the antioxidant acting as chain-breaking should effectively scavenge both the AOO$^•$ and AO$^•$ radicals. In case of these two *O*-centered radicals, although their standard reduction potential is lower than that for hydroxyl radical $^•$OH, H^+/H_2O (+2.31 V) [56], they are still very oxidative—similar to those of an aliphatic peroxyl radical ROO$^•$, H^+/ROOH (≈ +1.0 V) or an aliphatic alkoxyl radical RO$^•$, H^+/ROH (≈ +1.6 V) [57–59].

3.2. Assessment the Substance Antioxidative Profile by Hyaluronan Plus the Weissberger System

The idea to use high-molar-mass HA for the evaluation of antioxidants can be traced to the year 1994 [60,61]. At present, the experimental set-up uses the physiologic solution of the HA sample with an average molar mass ≈ 1.5 MDa. The time-dependent changes in

dynamic-viscosity values after the application of $CuCl_2$ and ascorbic acid are monitored by a rotation viscometry device [62]. In the experimental design when the application of the test substance precedes the addition of ascorbic acid, one examines the preventive antioxidant properties of the substance. On assessing the substance chain-breaking antioxidant properties the addition of substance examined follows the application of ascorbic acid after a properly selected time interval, i.e., during the steady state phase of HA degradation propagation. The panels in Figure 3 schematically illustrate the types of functional time dependencies on dynamic viscosity (η). (Other methods used were infrared spectroscopy [34,35], thermal chemiluminescence [34,35,63] and EPR spectroscopy [35,64], the last one to identify the formation of radicals in selected oxidation systems.) To comment the below represented experimental results it should be claimed that, as repeatedly proved [15–18,45,51,65,66], the decrease in dynamic viscosity values reflects the decrease in average molar mass of the HA sample with respect to the existing functional dependence η = f (M).

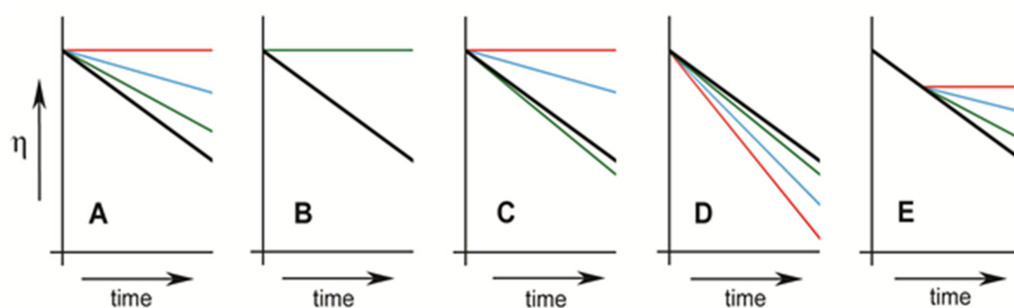


Figure 3. Panels (**A**–**D**) represent the results of assessing the substance functioning as a preventive antioxidant. Panel (**E**) schematically shows the η functional dependence observed when applying a chain-breaking antioxidant.

The time dependence of the decrease in the value of dynamic viscosity marked in black on panels A to E of the experimental set-up is as follows: $CuCl_2$ and ascorbic acid solution are gradually applied to the HA solution so that the actual concentrations of these components are 2 mg/mL, 1 and 100 µmol/L, respectively. It should also be noted here that the experiments were performed under aerobic conditions, i.e., the concentration of oxygen in aqueous solutions, at standard barometric pressure has been ≈250 µmol/L at 25 °C. When verifying whether a given substance acts as a preventive antioxidant, it was applied in several doses, so that the actual concentration of the substance within the experimental vessel was usually 100 (red), 10 (blue), and 1 (green curve) µmol/L, respectively. The results of antioxidative profile assessment of several substances are given in Table 1.

Table 1. Antioxidative profile assessment of several substances.

Substance	Preventive Action	Chain-Breaking Action	B	C	D	Reference/Note
N-Acetylcysteine	Effective	Effective		Effective [a]		[67–69]
Acetylsalicylic acid	Effective	Effective				[70]
Arbutin	Effective	Effective				[71]
Aurothiomalate	Effective	Effective				[72]
Bucillamine	Effective	Effective				[63,73–77]/It is a dithiol.
Captopril	Effective	Effective		Effective		[56,78]
Carnosine	Effective	Effective				[79]
Cemtirestat	Effective	Effective	Effective [b]			[59]
Cysteamine	Effective	Effective				[80]
L-Cysteine	Effective	Effective		Effective [a]		[73,81]
Dithiothreitol	Effective	Effective				[77]/It is a dithiol.
Dithioerythritol	Effective	Effective				[77]/It is a dithiol; SH groups' pK_a values = 9.2 and 10.1.
D-Mannitol	Effective	Effective				[61]
Edaravone	Effective	Effective				[82]
Ergothioneine	Effective	Effective				[83]
Isatin	Effective	Effective	Effective [b]			[59]
L-Glutathione	Effective	Effective		Effective [a]		[15,35,67,84–86]
Glutathione disulfide	Effective	Effective				[67]/It is the oxidized L-glutathione.
Hercynine	Ineffective	Ineffective				[83]
Histidine	Effective	Ineffective				[83]
Homocysteine	Effective	Effective				At neutral pH it is a zwitterion.
Ibuprofen	Effective	Not investigated				[87]/RS-(±)-, R- and S-enantiomers were evaluated.
Levamisole	Ineffective	Ineffective				[78]

Table 1. *Cont.*

Substance	Preventive Action	Chain-Breaking Action	B	C	D	Reference/Note
Methotrexate	Effective	Effective				[79]
Methylsulfonylmethane	Ineffective	Effective				[88]/Chain-breaker in extremely high dose.
Mn(III)-porphyrins [c]					Effective [c]	[89]
Naproxen	Effective	Effective				[70]
D-Penicillamine	Ineffective	Effective				[90,91]/SH group's pK_a value = 10.5
Phosphatidylcholine dihydroquercetin	Ineffective	Ineffective				[92]
Stobadine	Effective					[59,93,94]
Taxifolin	Ineffective	Ineffective				Dihydroquercetin, belongs to a subgroup of flavanols
Tiopronin	Effective	Effective				[56,78]
Vanillin	Effective	Effective				Phenol-type compound
Vinpocetine	Effective	Effective				[61]

[a] *N*-Acetylcysteine, L-cysteine, and L-glutathione are weak acids, which pK_a values of their −SH group equal to 9.52, 10.78, and 9.65, respectively. Due to dissociation of thiol a minute fraction of thiolate anions along with the generated thiyl radicals act both like the reductants of O_2 molecules yielding superoxide anion radicals, which subsequently participate in reaction with in situ generated H_2O_2 molecules according to the reversible Haber-Weiss reaction $O_2^{\bullet-} + H_2O_2 \leftrightarrow HO^{\bullet} + HO^{-} + O_2$ [95]. [b] Since both substances, i.e., cemtirestat and isatin strongly intercalate cupric ions, no redox active Cu(II) was available to form ascorbate—Cu(II)/Cu(I)—dioxygen complex even at the lowest substance concentration (1/100). [c] Mn(III)meso-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin (MnTE-2-PyP^{5+}), in phase II clinical trials, accepts one electron from $AscH^-$ and subsequently the Mn(II) center acts as a reductant of O_2 molecules yielding superoxide anion radical, which subsequently participate in reaction with in situ generated H_2O_2 molecules according to the above mentioned reversible Haber-Weiss reaction.

4. Concluding Remarks

We have reported evidence to support our thesis regarding the assessment of the antioxidative efficiency of an endo- or exogenous water soluble substance by exploiting a reaction system comprising high-molar-mass hyaluronan along with cupric cations and ascorbate. The ascorbate level in body fluids of healthy individuals is in the range of 40–140 μmol/L [50]. The total concentrations of copper ions in healthy human beings may reach micromolar levels [96]. Thus during ascorbate (auto)-oxidation in the presence of trace levels of cupric ions as catalysts, direct transformation of O_2 to H_2O_2 happens [7,8,10–13]. Subsequently, the produced hydrogen peroxide is decomposed by the action of the transition metal counter cuprous cations, which are site-specifically fixed by the HA polyanionic chain and thus $^{\bullet}OH$ radicals interact with HA macromolecules. The generated hydroxyl radicals, and/or mainly the *C*-centered hyaluronan macroradicals, if not scavenged by a proper preventive antioxidant, continue their subsequent self-perpetuating free-radical degradation up to the moment of their reaction with a chain-breaking antioxidant.

Author Contributions: Conceptualization, L.Š.; investigation, L.Š.; writing—original draft preparation, L.Š.; writing—K.V. and L.Š.; visualization, K.V. and L.Š.; supervision, K.V. and L.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by VEGA grant 2/0019/19 and by APVV PP-COVID-20-0043 grant.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study is openly available.

Acknowledgments: This research was funded by the grant VEGA 2/0019/19 and PP-COVID-20-0043. The authors are highly indebted to Professor Marian Valko for his critical reviewing of this communication.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Free Radical School Presentations. Available online: https://sfrbm.org/education/frs-presentations/ (accessed on 9 August 2021).
2. Shukla, N.; Maher, J.; Masters, J.; Angelini, G.D.; Jeremy, J.Y. Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? *Atherosclerosis* **2006**, *187*, 238–250. [CrossRef]
3. Aaseth, J.; Munthe, E.; Førre, Ø.; Steinnes, E. Trace Elements in Serum and Urine of Patients with Rheumatoid Arthritis. *Scand. J. Rheumatol.* **1978**, *7*, 237–240. [CrossRef]
4. Rafter, G. Plasma thiols, copper and rheumatoid arthritis. *Med. Hypotheses* **1994**, *43*, 59–61. [CrossRef]
5. Valko, M.; Morris, H.; Cronin, M.T.D. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* **2005**, *12*, 1161–1208. [CrossRef]
6. Udenfriend, S.; Clark, C.T.; Axelrod, J.; Brodie, B.B. Ascorbic acid in aromatic hydroxylation. I. A model system for aromatic hydroxylation. *J. Biol. Chem.* **1954**, *208*, 731–739. [CrossRef]
7. Weissberger, A.; LuValle, J.E.; Thomas, D.S., Jr. Oxidation processes. XVI. The autoxidation of ascorbic acid. *J. Am. Chem. Soc.* **1943**, *65*, 1934–1939. [CrossRef]
8. Khan, M.M.T.; Martell, A.E. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalyzed oxidation. *J. Am. Chem. Soc.* **1967**, *89*, 4176–4185. [CrossRef]
9. Wong, F.S.; Halliwell, B.; Richmond, R.; Skowroneck, W.R. The role of superoxide and hydroxyl radicals in the degradation of hyaluronic acid induced by metal ions and by ascorbic acid. *J. Inorg. Biochem.* **1981**, *14*, 127–134. [CrossRef]
10. Fisher, A.E.O.; Naughton, D.P. Iron supplements: The quick fix with long-term consequences. *Nutr. J.* **2004**, *3*, 2. [CrossRef]
11. Fisher, A.; Naughton, D. Vitamin C contributes to inflammation via radical generating mechanisms: A cautionary note. *Med. Hypotheses* **2003**, *61*, 657–660. [CrossRef]
12. Fisher, A.E.O.; Naughton, D.P. Therapeutic chelators for the twenty first century: New treatments for iron and copper mediated inflammatory and neurological disorders. *Curr. Drug Deliv.* **2005**, *2*, 261–268. [CrossRef]
13. Fisher, A.E.O.; Naughton, D.P. Why nutraceuticals do not prevent or treat Alzheimer's disease. *Nutr. J.* **2005**, *4*, 14. [CrossRef]
14. Šoltés, L.; Mendichi, R.; Kogan, G.; Schiller, J.; Stankovská, A.M.; Arnhold, J. Degradative action of reactive oxygen species on hyaluronan. *Biomacromolecules* **2006**, *7*, 659–668. [CrossRef]

15. Valachová, K.; Tamer, T.M.; Eldin, M.M.; Šoltés, L. Radical-scavenging activity of glutathione, chitin derivatives and their combination. *Chem. Pap.* **2016**, *70*. [CrossRef]
16. Valachová, K.; Topoľská, D.; Mendichi, R.; Collins, M.N.; Sasinková, V.; Šoltés, L. Hydrogen peroxide generation by the Weissberger biogenic oxidative system during hyaluronan degradation. *Carbohydr. Polym.* **2016**, *148*, 189–193. [CrossRef]
17. Buettner, G.; Jurkiewicz, B.A. Ascorbate free radical as a marker of oxidative stress: An EPR study. *Free Radic. Biol. Med.* **1993**, *14*, 49–55. [CrossRef]
18. Šoltés, L.; Kogan, G.; Stankovská, M.; Mendichi, R.; Rychlý, J.; Schiller, A.J.; Gemeiner, P. Degradation of high-molar-mass hyaluronan and characterization of fragments. *Biomacromolecules* **2007**, *8*, 2697–2705. [CrossRef]
19. Fornaro, A.; Coichev, N. Ácido L-ascórbico: Reações de complexação e de óxido-redução com alguns íons metálicos de transição. *Divulgação* **1998**, *21*, 642–650. [CrossRef]
20. Lepperdinger, G.; Fehrer, C.; Reitinger, S. Chemistry and biology of hyaluronan. In *Chemistry and Biology of Hyaluronan*; Garg, H.G., Hales, C.A., Eds.; Elsevier Press: Amsterdam, NY, USA, 2004; pp. 71–82.
21. Stern, R.; Asari, A.A.; Sugahara, K.N. Hyaluronan fragments: An information-rich system. *Eur. J. Cell. Biol.* **2006**, *85*, 699–715. [CrossRef]
22. Mendichi, R.; Šoltés, L. Hyaluronan molecular weight and polydispersity in some commercial intra-articular injectable preparations and in synovial fluid. *Inflamm. Res.* **2002**, *51*, 115–116. [CrossRef]
23. Šoltés, L.; Valachová, K. Biopolymer hyaluronan messenging the status of synovial joints. In *Advances in Chemistry Research*; Taylor, J.C., Ed.; Nova Science Publishers, Inc.: Hauppauge, NY, USA, 2018; pp. 157–180.
24. Valachová, K.; Šoltés, L. Versatile use of chitosan and hyaluronan in medicine. *Molecules* **2021**, *26*, 1195. [CrossRef]
25. Nitzan, D.W.; Nitzan, U.; Dan, P.; Yedgar, S. The role of hyaluronic acid in protecting surface-active phospholipids from lysis by exogenous phospholipasae A2. *Rheumatology* **2001**, *40*, 336–340. [CrossRef]
26. Kogan, G.; Šoltés, L.; Stern, R.; Gemeiner, P. Hyaluronic acid: A natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol. Lett.* **2007**, *29*, 17–25. [CrossRef]
27. Furth, G.; Knierim, R.; Buss, V.; Mayer, C. Binding of bivalent cations by hyaluronate in aqueous solution. *Int. J. Biol. Macromol.* **2008**, *42*, 33–40. [CrossRef]
28. Rinaudo, M.; Rozand, Y.; Mathieu, P.; Conrozier, T. Role of different pre-treatments on composition and rheology of synovial fluids. *Polymers* **2009**, *1*, 16–34. [CrossRef]
29. Volpi, N.; Schiller, J.; Stern, R.; Šoltés, L. Role, metabolism, chemical modifications and applications of hyaluronan. *Curr. Med. Chem.* **2009**, *16*, 1718–1745. [CrossRef]
30. Schiller, J.; Volpi, N.; Hrabarová, E.; Šoltés, L. Hyaluronic acid: A natural biopolymer. In *Handbook of Biopolymers and Their Applications*; Kalia, S., Averous, L., Eds.; Wiley & Scrivener Publishing: Beverly, MA, USA, 2011; pp. 3–34.
31. Valachová, K.; Volpi, N.; Stern, R.; Šoltés, L. Hyaluronan in medical practice. *Curr. Med. Chem.* **2016**, *23*, 3607–3617. [CrossRef]
32. Bishop, P.N. Structural macromolecules and supramolecular organisation of the vitreous gel. *Prog. Retin. Eye Res.* **2000**, *19*, 323–344. [CrossRef]
33. Nickerson, C.S.; Kornfield, J.A.A. "Cleat" geometry for suppressing wall slip. *J. Rheol.* **2005**, *49*, 865–874. [CrossRef]
34. Rychlý, J.; Šoltés, L.; Stankovská, M.; Janigová, I.; Csomorová, K.; Sasinková, V.; Kogan, G.; Gemeiner, P. Unexplored capabilities of chemiluminescence and thermoanalytical methods in characterization of intact and degraded hyaluronans. *Polym. Degrad. Stab.* **2006**, *91*, 3174–3184. [CrossRef]
35. Hrabárová, E.; Valachová, K.; Rychlý, J.; Rapta, P.; Sasinková, V.; Malíková, M.; Šoltés, L. High-molar-mass hyaluronan degradation by Weissberger's system: Pro- and anti-oxidative effects of some thiol compounds. *Polym. Degrad. Stab.* **2009**, *94*, 1867–1875. [CrossRef]
36. Collins, M.N.; Birkinshaw, C. Hyaluronic acid based scaffolds for tissue engineering—A review. *Carbohydr. Polym.* **2013**, *92*, 1262–1279. [CrossRef]
37. Chabreček, P.; Šoltés, L.; Kállay, Z.; Fügedi, A. Isolation and characterisation of high moleculr weight (3H) hyaluronic acid. *J. Label. Comp. Radiopharm.* **1990**, *28*, 1121–1125. [CrossRef]
38. Chabreček, P.; Šoltés, L.; Orviský, E. Comparative depolymerization of sodium hyaluronate by ultrasonic and enzymatic treatments. *J. App. Polymer Sci. App. Polym. Symp.* **1991**, *48*, 233–241. [CrossRef]
39. Chabreček, P.; Šoltés, L.; Hradec, H.; Filip, J.; Orviský, E. Preparation and characterization of the high molecular weight (3H) hyaluronic acid. *Collect. Czechoslov. Chem. Commun.* **1992**, *57*, 2151–2156. [CrossRef]
40. Orviský, E.; Šoltés, L.; Chabreček, P.; Novák, I.; Kéry, V.; Stančíková, M.; Vinš, I. The determination of hyaluronan molecular weight distribution by means of high-performance size exclusion chromatography. *J. Liq. Chromatogr.* **1992**, *15*, 3203–3218. [CrossRef]
41. Orviský, E.; Šoltés, L.; Chabreček, P.; Novák, I.; Stančíková, M. Size exclusion chromatographic characterization of sodium hyaluronate fractions prepared by high energetic sonication. *Chromatographia* **1993**, *37*, 20–22. [CrossRef]
42. Laurent, T.C.; Laurent, U.B.; Fraser, J.R. Serum hyaluronan as disease marker. *Ann. Med.* **1996**, *28*, 241–253. [CrossRef]
43. Fraser, J.R.; Laurent, T.C.; Laurent, U.B. Hyaluronan: Its nature, distribution, functions and turnover. *J. Int. Med.* **1997**, *242*, 27–33. [CrossRef]

44. Grootveld, M.; Henderson, E.B.; Farrell, A.; Blake, D.R.; Parkes, H.G.; Haycock, P. Oxidative damage to hyaluronate and glucose in synovial fluid during exercise of the inflamed rheumatoid joint: Detection of abnormal low-molecular-mass metabolites by proton-n.m.r. spectroscopy. *Biochem. J.* **1991**, *273*, 459–467. [CrossRef]
45. Šoltés, L.; Stankovská, M.; Brezová, V.; Schiller, J.; Arnhold, J.; Kogan, G.; Gemeiner, P. Hyaluronan degradation by copper(II) chloride and ascorbate: Rotational viscometric, EPR spin-trapping, and MALDI-TOF mass spectrometric investigations. *Carbohydr. Res.* **2006**, *341*, 2826–2834. [CrossRef]
46. Juránek, I.; Šoltés, L. Reactive oxygen species in joint physiology: Possible mechanism of maintaining hypoxia to protect chondrocytes from oxygen excess via synovial fluid hyaluronan peroxidation. In *Kinetics, Catalysis and Mechanism of Chemical Reactions: From Pure to Applied Science*; Islamova, R.M., Kolesov, S.V., Zaikov, G.E., Eds.; Nova Science Publishers: New York, NY, USA, 2012; pp. 1–10.
47. Juránek, I.; Stern, R.; Šoltés, L. Hyaluronan peroxidation is required for normal synovial function: An hypothesis. *Med. Hypotheses* **2014**, *82*, 662–666. [CrossRef]
48. Stern, R.; Kogan, G.; Jedrzejas, M.J.; Šoltés, L. The many ways to cleave hyaluronan. *Biotechnol. Adv.* **2007**, *25*, 537–557. [CrossRef]
49. Koch, P.; Sidloi, M.; Tonks, D.B. Estimation of serum ascorbic acid in patients and the effect of ascorbic acid and its oxidation products on SMA 12/60 parameters. *Clin. Biochem.* **1980**, *13*, 73–77. [CrossRef]
50. Halliwell, B.; Wasil, M.; Grootveld, M. Biologically significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by ascorbic acid. Implications for antioxidant protection in the inflamed rheumatoid joint. *FEBS Lett.* **1987**, *213*, 15–17. [CrossRef]
51. Šoltés, L.; Valachová, K.; Mendichi, R.; Kogan, G.; Arnhold, J.; Gemeiner, P. Solution properties of high-molar-mass hyaluronans: The biopolymer degradation by ascorbate. *Carbohydr. Res.* **2007**, *342*, 1071–1077. [CrossRef] [PubMed]
52. Hawkins, C.L.; Davies, M.J. Detection of intermediates formed on reaction of hyaluronic acid and related materials with the hydroxyl radical. *Biochem. Soc. Trans.* **1995**, *23*, S248. [CrossRef]
53. Hawkins, C.L.; Davies, M.J. Direct detection and identification of radicals generated during the hydroxyl radical-induced degradation of hyaluronic acid and related materials. *Free Radic. Biol. Med.* **1996**, *21*, 275–290. [CrossRef]
54. Kogan, G.; Šoltés, L.; Stern, R.; Mendichi, R. Hyaluronic acid: A biopolymer with versatile physico-chemical and biological properties. In *Handbook of Polymer Research: Monomers, Oligomers, Polymers and Composites*; Pethrick, R.A., Ballada, A., Zaikov, G.E., Eds.; Nova Science Publishers: New York, NY, USA, 2007; pp. 393–439.
55. Kogan, G.; Šoltés, L.; Stern, R.; Schiller, J.; Mendichi, R. *Studies in Natural Products Chemistry. Hyaluronic Acid: Its Function and Degradation in In Vivo Systems*; Elsevier: Amsterdam, The Netherland, 2008; pp. 789–882.
56. Valachova, K.; Svik, K.; Biro, C.; Soltes, L. Skin wound healing with composite biomembranes loaded by tiopronin or captopril. *J. Biotechnol.* **2020**, *310*, 49–53. [CrossRef] [PubMed]
57. Buettner, G.R. The pecking order of free radicals and antioxidants: Lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* **1993**, *300*, 535–543. [CrossRef]
58. Buettner, G.R.; Schafer, F.Q. Ascorbate (vitamin C) as an antioxidant. In *Vitamin C: Its Functions and Biochemistry in Animals and Plants*; May, J.M., Asard, H., Smirnoff, N., Eds.; BIOS Scientific Publishers: Oxford, UK, 2004; pp. 173–188.
59. Valachová, K.; Mach, M.; Šoltés, L. Oxidative degradation of high-molar-mass hyaluronan: Effects of some indole derivatives to hyaluronan decay. *Int. J. Mol. Sci.* **2020**, *20*, 5609. [CrossRef]
60. Orviský, E.; Šoltés, L.; Stanèíková, M.; Vyletelová, Z.; Juránek, I. Assessment of Antioxidative Properties of Hydrophilic Xenobiotics on the Basis of Inhibition of the Radical Degradation of Hyaluronan by Reactive Oxygen Species. Slovak Patent No. 2764, 6 June 1994.
61. Orviský, E.; Šoltés, L.; Stanèíková, M. High-molecular-weight hyaluronan—A valuable tool in testing the antioxidative activity of amphiphilic drugs stobadine and vinpocetine. *J. Pharm. Biomed. Anal.* **1997**, *16*, 419–424. [CrossRef]
62. Stankovská, M.; Šoltés, L.; Vikartovská, A.; Mendichi, R.; Láth, D.; Molnárová, M.; Gemeiner, P. Study of hyaluronan degradation by means of rotational viscometry: Contribution of the material of viscometer. *Chem. Pap.* **2004**, *58*, 348–352.
63. Baòasová, M.; Valachová, K.; Rychlý, J.; Janigová, I.; Csomorová, K.; Mendichi, R.; Mislovièová, D.; Juránek, I.; Šoltés, L. Free-radical-mediated degradation of high-molar-mass hyaluronan. Action of an antiinflammatory drug. *Polymers* **2014**, *6*, 2625–2644. [CrossRef]
64. Topoľská, D.; Valachová, K.; Rapta, P.; Šilhár, S.; Panghyová, E.; Horváth, A.; Šoltés, L. Antioxidative properties of Sambucus Nigra extracts. *Chem. Pap.* **2015**, *69*, 1202–1210. [CrossRef]
65. Mendichi, R.; Šoltés, L.; Schieroni, A.G. Evaluation of radius of gyration and intrinsic viscosity molar mass dependence and stiffness of hyaluronan. *Biomacromolecules* **2003**, *4*, 1805–1810. [CrossRef]
66. Valachova, K.; Topolska, D.; Mendichi, R.; Rychly, J.; Soltes, L. Protective effects of catalase indicate hydrogen peroxide involvement in hyaluronan degradation initiated by Cu(II) ions plus ascorbate in situ. In *Applied Chemistry and Chemical Engineering, Principles, Methodology, and Evaluation Methods*; Haghi, A.K., Pogliani, L., Balkose, D., Mukbaniani., O.V., Mercader, A.G., Eds.; Apple Academic Press: Waretown, NJ, USA; Taylor & Francis Group: Palm Bay, FL, USA, 2018; Volume 2, pp. 157–173.
67. Valachová, K.; Šušaníková, I.; Topoľská, D.; Bögi, E.; Šoltés, L. Effects of N-acetylcysteine, glutathione, and glutathione disulfide on NIH 3T3, VH10 and MCF-7 cells exposed to ascorbate and Cu(II) ions. In *Green Chemistry and Sustainable Technology. Biological, Pharmaceutical, And Macromolecular Systems*; Dake, S.A., Shinde, R.S., Ameta, S.C., Haghi, A.K., Eds.; Apple Academic Press: Waretown, NJ, USA; Taylor & Francis Group: Palm Bay, FL, USA, 2021; pp. 77–93.

68. Hrabárová, E.; Rychlý, J.; Sasinková, V.; Valachová, K.; Janigová, I.; Csomorová, K.; Juránek, I.; Šoltés, L. Structural characterisation of thiol-modified hyaluronans. *Cellulose* **2012**, *19*, 2093–2104. [CrossRef]
69. Hrabarova, E.; Valachova, K.; Juranek, I.; Soltes, L. Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions: Evaluation of antioxidative effect of cysteine derived compounds. *Chem. Biodivers.* **2012**, *9*, 309–317. [CrossRef]
70. Šoltés, L.; Stankovská, M.; Kogan, G.; Mendichi, R.; Volpi, N.; Sasinková, V.; Gemeiner, P. Degradation of high-molar-mass hyaluronan by an oxidative system comprising ascorbate, Cu(II), and hydrogen peroxide: Inhibitory action of antinflammatory drugs—Naproxen and acetylsalicylic acid. *J. Pharm. Biomed. Anal.* **2007**, *44*, 1056–1063. [CrossRef]
71. Valachová, K.; Rapta, P.; Slováková, M.; Priesolová, E.; Nagy, M.; Mislovičová, D.; Dráfi, F.; Bauerová, K.; Šoltés, L. Radical degradation of high molar mass hyaluronan induced by ascorbate plus cupric ions. Testing of arbutin in the function of antioxidant. In *Advances in Kinetics and Mechanism of Chemical Reactions*; Zaikov, G.E., Valente, A.J.M., Iordanskii, A.L., Eds.; Apple Academic Press: Waretown, NJ, USA, 2013; pp. 1–19.
72. Valachová, K.; Vargová, A.; Rapta, P.; Hrabárová, E.; Dráfi, F.; Bauerová, K.; Juránek, I.; Šoltés, L. Aurothiomalate as preventive and chain-breaking antioxidant in radical degradation of high-molar-mass hyaluronan. *Chem. Biodivers.* **2011**, *8*, 1274–1283. [CrossRef]
73. Valachová, K.; Hrabárová, E.; Priesolová, E.; Nagy, M.; Baňasová, M.; Juránek, I.; Šoltés, L. Free-radical degradation of high-molecular-weight hyaluronan induced by ascorbate plus cupric ions. Testing of bucillamine and its SA981-metabolite as antioxidants. *J. Pharm. Biomed. Anal.* **2011**, *56*, 664–670. [CrossRef] [PubMed]
74. Baňasová, M.; Valachová, K.; Hrabárová, E.; Priesolová, E.; Nagy, M.; Juránek, I.; Šoltés, L. Early stage of the acute phase of joint inflammation in vitro testing of bucillamine and its oxidized metabolite SA981 in function of antioxidants. *Interdis. Toxicol.* **2011**, *4*, A22.
75. Baňasová, M.; Sasinková, V.; Mendichi, R.; Perečko, T.; Valachová, K.; Juránek, I.; Šoltés, L. Free-radical degradation of high-molar-mass hyaluronan induced by Weissberger's oxidative system: Potential antioxidative effect of bucillamine. *Neuroendocrinol. Lett.* **2012**, *3* (Suppl. S33), 151–154.
76. Baňasová, M.; Kerner, L.; Juránek, I.; Putala, M.; Valachová, K.; Šoltés, L. Radical scavenging capacity of *N*-(2-mercapto-2-methylpropionyl)-l-cysteine. Design and synthesis of its derivative with enhanced potential to scavenge hypochlorite. *J. Inf. Intell. Knowl.* **2014**, *6*, 453–470.
77. Baňasová, M.; Valachová, K.; Juránek, I.; Šoltés, L. Dithiols as more effective than monothiols in protecting biomacromolecules from free-radical-mediated damage: In vitro oxidative degradation of high-molar-mass hyaluronan. *Chem. Pap.* **2014**, *68*, 1428–1434. [CrossRef]
78. Valachová, K.; Baňasová, M.; Topoľská, D.; Sasinková, V.; Juránek, I.; Collins, M.N.; Šoltés, L. Influence of tiopronin, captopril and levamisole therapeutics on the oxidative degradation of hyaluronan. *Carbohydr. Polym.* **2015**, *134*, 516–523. [CrossRef]
79. Dráfi, F.; Bauerová, K.; Valachová, K.; Poništ, S.; Mihálová, D.; Juránek, I.; Boldyrev, A.; Hrabárová, E.; Šoltés, L. Carnosine inhibits degradation of hyaluronan induced by free radical processes in vitro and improves the redox imbalance in adjuvant arthritis in vivo. *Neuroendocrinol. Lett.* **2010**, *31* (Suppl. S2), 96–100. [PubMed]
80. Valachová, K.; Hrabárová, E.; Dráfi, F.; Juránek, I.; Bauerová, K.; Priesolová, E.; Nagy, M.; Šoltés, L. Ascorbate and Cu(II) induced oxidative degradation of high-molar-mass hyaluronan. Pro- and antioxidative effects of some thiols. *Neuroendocrinol. Lett.* **2010**, *31* (Suppl. S2), 101–104.
81. Valent, I.; Topoľská, D.; Valachová, K.; Bujdák, J.; Šoltés, L. Kinetics of ABTS derived radical cation scavenging by bucillamine, cysteine, and glutathione. Catalytic effect of Cu^{2+} ions. *Biophys. Chem.* **2016**, *212*, 9–16. [CrossRef]
82. Tamer, T.M.; Valachová, K.; Hassan, M.A.; Omer, A.M.; El-Shafeey, M.; Eldin, M.M.; Šoltés, L. Chitosan/hyaluronan/edaravone membranes for anti-inflammatory wound dressing: In vitro and in vivo evaluation studies. *Mater. Sci. Eng. C* **2018**, *90*, 227–235. [CrossRef]
83. Valachová, K.; Švík, K.; Biró, C.; Collins, M.N.; Jurčík, R.; Ondruška, L.; Šoltés, L. Impact of ergothioneine, hercynine and histidine on oxidative degradation of hyaluronan and wound healing. *Polymers* **2021**, *13*, 95. [CrossRef]
84. Valachová, K.; Mendichi, R.; Šoltés, L. *Effect of L-Glutathione on High-Molar-Mass Hyaluronan Degradation by Oxidative System Cu(II) Plus Ascorbate. Monomers, Oligomers, Polymers, Composites, and Nanocomposites*; Pethrick, R.A., Petkov, P., Zlatarov, A., Zaikov, G.E., Rakovsky, S.K., Eds.; Nova Science Publishers: New York, NY, USA, 2010; pp. 101–111.
85. Tamer, M.T.; Valachová, K.; Omer, A.M.; Sabet, M.M.; Šoltés, L. Effects of glutathione, phosphonate, or sulfonated chitosans and their combination on scavenging free radicals. In *High-Performance Materials and Engineered Chemistry*; Torrens, F., Balkose, D., Thomas, S., Eds.; Apple Academic Press: New Jersey, NJ, USA, 2018; pp. 371–389.
86. Tamer, M.T.; Hassan, M.A.; Valachová, K.; Omer, A.M.; El-Shafeey, M.E.A.; Mohy Eldin, M.S.; Šoltés, L. Enhancement of wound healing by chitosan/hyaluronan polyelectrolyte membrane loaded with glutathione: In vitro and in vivo evaluations. *J. Biotechnol.* **2020**, *310*, 103–113. [CrossRef]
87. Šoltés, L.; Láth, D.; Mendichi, R.; Bystrický, P. Radical degradation of high molecular weight hyaluronan: Inhibition of the reaction by ibuprofen enantiomers. Method and Find. *Exp. Clin. Pharmacol.* **2001**, *23*, 65–71.
88. Baňasová, M.; Valachová, K.; Juránek, I.; Šoltés, L. Aloe vera and methylsulfonylmethane as dietary supplements: Their potential benefits for arthritic patients with diabetic complications. *J. Inf. Intell. Knowl.* **2013**, *5*, 51–68.

89. Valachová, K.; Rapta, P.; Moura, N.M.M.; Batinic-Haberle, I.; Šoltés, L. Ortho isomeric Mn(III) N-alkyl- and alkoxyalkylpyridylporphyrins—enhancers of hyaluronan degradation induced by ascorbate and cupric ions. *Int. J. Mol. Sci.* **2021**, *22*, 8608. [CrossRef]
90. Valachová, K.; Hrabárová, E.; Gemeiner, P.; Šoltés, L. Study of pro- and anti-oxidative properties of D-penicillamine in a system comprising high-molar-mass hyaluronan, ascorbate, and cupric ions. *Neuroendocrinol. Lett.* **2008**, *29*, 697–701. [PubMed]
91. Valachová, K.; Rapta, P.; Kogan, G.; Hrabárová, E.; Gemeiner, P.; Šoltés, L. Degradation of high-molar-mass hyaluronan by ascorbate plus cupric ions: Effects of D-penicillamine addition. *Chem. Biodivers.* **2009**, *6*, 389–395. [CrossRef] [PubMed]
92. Hassan, M.A.; Tamer, M.T.; Valachová, K.; Omer, A.M.; El-Shafeey, M.; Mohy Eldin, M.S.; Šoltés, L. Antioxidant and antibacterial polyelectrolyte wound dressing based on chitosan/hyaluronan/phosphatidylcholine dihydroquercetin. *Int. J. Biol. Macromol.* **2021**, *166*, 18–31. [CrossRef]
93. Rapta, P.; Valachová, K.; Zalibera, M.; Šnirc, V.; Šoltés, L. Hyaluronan degradation by reactive oxygen species: Scavenging effect of the hexahydropyridoindole stobadine and two of its derivatives. In *Monomers, Oligomers, Polymers, Composites, and Nanocomposites*; Pethrick, A.R., Petkov, P., Zlatarov, A., Zaikov, G.E., Rakovsky, S.K., Eds.; Nova Science Publishers: New York, NY, USA, 2010; pp. 113–126.
94. Surovčíková, L.; Valachová, K.; Baňasová, M.; Šnirc, V.; Priesolová, E.; Nagy, M.; Juránek, I.; Šoltés, L. Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions: Testing of stobadine and its two derivatives in function as antioxidants. *Gen. Physiol. Biophys.* **2012**, *31*, 57–64. [CrossRef] [PubMed]
95. Oikawa, S.; Yamada, K.; Yamashita, N.; Tada-Oikawa, S.; Kawanishi, S. N-Acetylcysteine, a cancer chemopreventive agent, causes oxidative damage to cellular and isolated DNA. *Carcinogenesis* **1999**, *20*, 1485–1490. [CrossRef]
96. Koppenol, W.H. Chemistry of iron and copper in radical reactions. In *Free Radical Damage and Its Control*; Rice-Evans, C.A., Burdon, R.H., Eds.; Elsevier: Amsterdam, The Netherlands, 1994; pp. 3–24.