Radical Degradation of High Molecular Weight
Hyaluronan: Inhibition of the Reaction by Ibuprofen Enantiomers*

Institute of Experimental Pharmacology and 1Polymer Institute, Slovak
Academy of Sciences, Bratislava, Slovak Republic; 2Institute of Macromolecular
Chemistry, National Research Council, Milano, Italy

SUMMARY

The antioxidative and/or free-radical-scavenging activities of R-()- and S-(+)-
ibuprofen enantiomers, as well as of the drug racemate, were studied in vitro on
measuring the kinetics of (uninhibited or drug-inhibited) degradation of high
molecular weight hyaluronan by hydroxyl radicals. The continual flux of OH radicals
radicals at aerobic conditions was maintained by the $\text{H}_2\text{O}_2 + \text{Cu}^{2+}$ system. The
kinetics of hyaluronan degradation was monitored indirectly by capillary
viscometry. Under experimental conditions, with no drug addition, the relative
viscosity ($\theta_{rel}$) decreased continuously, reaching 13% of the initial $\theta_{rel}$ value in
in 4 h. Each drug tested exhibited a dose-dependent protective effect against
hyaluronan degradation, however R-()-ibuprofen demonstrated a slightly greater
activity than the drug S-(+)-enantiomer. © 2001 Prous Science. All rights reserved.

Key words: Arthritic diseases - Biopolymers - Capillary viscometry -
Glycosaminoglycans - Hyaluronan degradation - Hydroxyl radicals - Ibuprofen
enantiomers - Inflammatory arthritis - OH radicals

INTRODUCTION

One of the prominent features of inflammatory arthritis (osteoarthritis, rheumatoid arthritis) is the loss of viscosity of the synovial fluid, due to the
free/OH-radical- degradation of its major macromolecular component, hyaluronan (HA; Fig. 1). Protection of HA against OH-radical-induced
degradation has been confirmed in vitro with several types of pharmaceuticals including nonsteroidal antiinflammatory drugs (NSAIDs), antioxidants and free radical scavengers (4-6).
FIG. 1. Chemical structure of high molecular weight hyaluronan (acid form). The term hyaluronic acid, coined by K. Meyer in 1958 (1), was assigned to a nonbranched glycosaminoglycan, mucopolysaccharide, composed of regularly alternating units of D-glucuronic acid and N-acetyl-D-glucosamine linked by \( \beta-(1,4) \) and \( \alpha-(1,3) \) linkages. This glycosaminoglycan is present in all tissues and body fluids of vertebrates. Under physiological (pH) conditions, it is a polyanion (with corresponding counter-cation(s) such as \( \text{H}^+ \), \( \text{Na}^+ \), \( \text{K}^+ \), etc.) and hence the term hyaluronate appears to be more appropriate. However, irrespective of the degree of dissociation of its macrobiomolecules, the term hyaluronan, suggested by Bárány et al. in 1986 (2), has become predominantly used for the designation of this polysaccharide (3).

Ibuprofen (Fig. 2), classified as a chiral 2-arylpropionic acid from the group of NSAIDs, exists as \( R-(\text{--}) \) and \( S-(\text{++}) \)-enantiomer. The dextrorotatory \( S-(\text{+}) \)-ibuprofen has been reported to be about 160 times more effective than the levorotatory \( R-(\text{--}) \)-isomer in inhibiting prostaglandin synthesis \textit{in vitro} (7). Nevertheless, despite the well-established difference in the pain-blocking activity of the two enantiomers, ibuprofen has to date been marketed as the \( RS-(\text{±}) \)-racemic mixture in the majority of countries. This may also be accounted for by the fact that \textit{in vivo} (in human beings) the \( R-(\text{--}) \)-enantiomer is partially inverted to \( S-(\text{+}) \)-ibuprofen (8, 9).

FIG. 2. Chemical structure of ibuprofen, \( (RS)-(\text{±}) \)-2-(4-isobutyl-phenyl)-propionic acid. Ibuprofen was developed in the 1960s as a racemic antirheumatic and, since 1967 in the U.K. and 1974 in the U.S., it has been widely used as a prescription drug. It became available as an over-the-counter drug in these two countries in 1984. Today it is used in more than 100 countries. \( S-(\text{+}) \)-Ibuprofen (the product of Garbo, Fieberbrunn, Austria) has been available clinically in Austria since 1994 (7).

The ibuprofen dose ingested should be relatively high (400-1200 mg) since the drug binding to plasma (albumin) is, in a concentration-dependent manner, really extensive (>98%) and moreover, stereoselective; the \( S-(\text{+}) \)-isomer is more tightly bound to albumin, with its apparent binding constant equaling \( 8.0 \pm 0.11 \times 10^5 \text{l/mol} \) vs. \( 4.8 \pm 0.07 \times 10^5 \text{l/mol} \) found for \( R-(\text{--}) \)-ibuprofen (7). The total ibuprofen plasma concentration, \textit{i.e.}, the level of free + protein-bound drug fractions, has a therapeutic window between 10 to 50 mg/l; concentrations above 100 mg/l are considered toxic. At ingested drug doses exceeding 600 mg, there is an increase in the ibuprofen plasma unbound fraction (7).

On comparing plasma from healthy individuals to that of patients with rheumatoid arthritis, at (total) drug levels between 2 and 50 mg/l, ibuprofen appears to be less strongly bound to the rheumatic plasma. This fact, however, may be the consequence of lower albumin concentrations 51 g/l in normal vs. 46 g/l in rheumatic plasma. The drug protein binding in patients with rheumatoid arthritis and osteoarthritis demonstrated no significant difference between these groups.

Substantial ibuprofen concentrations should be attained in synovial fluid since the synovial membrane is the proposed primary site of the inhibitory action of NSAIDs on prostaglandin synthesis. Pharmacokinetic analysis suggested that the \( R-(\text{--}) \) and \( S-(\text{+}) \)-ibuprofen molecules enter the synovial fluid compartment in the unbound form. However the drug fate in this micro-environment, as well as the form in which the drug molecules diffuse out of the synovial fluid, is less clear (7).

The objective of this study was to gain better insight into the interaction(s) of
ibuprofen with artificial synovial fluid by studying the kinetics of inhibition of OH radical-induced degradation of high molecular weight hyaluronan by the individual enantiomers of ibuprofen.

MATERIALS AND METHODS

Drugs, biopolymers, chemicals

Ibuprofen racemate (C$_{13}$H$_{18}$O$_2$; m.w. = 206.3 Da) as well as both its R-(-) and S-(-) enantiomers with an optical purity of 100.0 and 99.6% were kindly supplied by Dr. D.P. Bauer, Director of the Ethyl Corporation (Baton Rouge, LA, USA).

High molecular weight hyaluronan a lyophilisate of the sodium hyaluronate salt was donated by the Contipro Chemical Company (Ústí nad Orlicí, Czech Republic). The molecular weight parameters determined for this HA sample were $M_w$ = 659.4 kDa, $M_w/M_n = 1.88$, and $M_z/M_w = 1.59$ (10). Another high molecular weight sodium hyaluronate (powderized) sample (U.S. Patent No. 4,517,295), $M_v = 1.2$ MDa, was kindly supplied by Dr. K. Thacker, Lifecore Biomedical Inc. (Chaska, MN, USA). The $M_n$, $M_v$, $M_w$, and $M_z$ represent the number-, viscosity-, weight-, and z-average of the (bio)polymer molecular weights.

CuSO$_4$ x 5H$_2$O, NaCl, both of p.a. purity grade, as well as the concentrated 30-% (w/v) hydrogen peroxide were purchased from Lachema, Brno, Czech Republic.

Stock solutions

The stock solvent used was an aqueous solution containing CuSO$_4$ (0.2 mmol/l) and NaCl (0.15 mol/l).

HA$_{stock}$ represents a 0.1-% (w/v) hyaluronan dissolved in the (above-specified) stock solvent. Thus, the sample with $M_w$ = 659.4 kDa had an HA$_{stock}$ concentration of 1.5 ?mol/l and for the sample with $M_v$ = 1.2 MDa it equaled 0.83 ?mol/l. The HA$_{stock}$ solutions, kept at 0°C in the dark, were stable for at least 5 days.

The (concentrated) RS-, R-, or S-stock solution was prepared by dissolving the corresponding RS-(±)-, R-(-), or S-(+)ibuprofen in the stock solvent. Since ibuprofen an organic acid is sparingly soluble in water (11.3 mg/l (11)), the drug was first converted to its Na-salt, which is well (water) soluble (12).

Working solutions

The working HA solution was prepared by diluting the HA$_{stock}$ with the stock solvent so as to obtain the relative viscosity ($[\eta]_{rel}$) = 2.1-2.2 (see Capillary viscometry). ($[\eta]_{rel}$ of the stock solvent = 1.0).

The working (HA + drug) solution was prepared by mixing the appropriate volumes of the above-specified stock solutions so as to obtain the actual HA concentration identical with the hyaluronan level set in the working HA solution and the $[\eta]_{rel}$ value of the working (HA + drug) solution = 2.1-2.2.

Capillary viscometry

The viscosity measurements were performed at 25.0 ± 0.05 °C by using an Ubbelohde dilution viscometer. The diameter of the viscometer capillary was 0.53 mm. The total run/flow-time of the stock solvent used ($[\eta]_{0}$) was 85.0 ± 0.1 sec.
The constant volume (10.0 ml) of the given working solution was loaded into the viscometer reservoir. After equilibrating the temperature, the flow-time ([?], i.e., the viscosity characteristic of the given solution) was measured. Then

100.0 ?l of the aqueous H₂O₂ solution (3.0-% (v/v)) was added to the working solution in the viscometer and, at selected time intervals (Fig. 3A and B), the solution flow-times (i.e., the kinetics of [?] decrease) were determined. The kinetics of the uninhibited/inhibited degradation of the high molecular weight HA sample was monitored for up to 4 h.

A.

---

B.

---
FIG. 3. A) Free radical scavenging activity of RS-(±)-ibuprofen on hyaluronan degradation. Drug concentration = 0.0 mg/l (0); or = 43.6; 31.3; 22.0; 9.6; or 5.1 mg/l (l). B) Free radical scavenging activity of R-( − )- or S-(+)-ibuprofen on hyaluronan degradation. Drug concentration = 0.0 mg/l (0); or = 43.6; 31.3; 22.0; 9.6; or 5.1 mg/l (right filled circle) R-( − ), or (left filled circle) S-(+)-ibuprofen.

The (initial) concentration of the working solution as well as the time during which the degradation kinetics was monitored were chosen so as to fulfill the following condition: $2.2 \times \left[ \frac{?}{?} \right]_{\text{rel.}} \times \left[ \frac{?}{?} \right]_0 \times \left[ \frac{1}{1.1} \right]$. Corrections on the kinetic energy of the flow of the solutions measured as well as the effect of shearing were omitted at this stage of the study.

RESULTS

As evident in Figure 3, after H$_2$O$_2$ addition the high molecular weight hyaluronan became extensively degraded. With prolonged exposure times the decrease in $[?]_{\text{rel.}}$ became more pronounced. Under the experimental conditions used, the relative viscosity of the working HA solution decreased significantly, reaching 13% of the initial $[?]_{\text{rel.}}$ value in 4 h. However, when 100.0 ?l of H$_2$O was added to the high molecular weight hyaluronan, instead of hydrogen peroxide, the stability of the $[?]_{\text{rel.}}$ was appropriate during the whole time interval up to 4 h.

On repeated measurements, satisfactory reproducibility was stated. The slightly greater values of the standard error of mean (SEM) evidenced at the early time intervals of the reaction (Fig. 3, bars at 1, 3, and 5 min) were exclusively due to the process of equilibration after addition of 100.0 ?l of H$_2$O$_2$ and its dispersion in the volume of 10.0 ml of the working solution. (The SEM values at the reaction onset (£10 min) are practically negligible; the bars are within the experimental points).

The measurements of the changes in $[?]_{\text{rel.}}$ of solutions with the addition of RS-(±)-ibuprofen (Fig. 3A) or with one single R-(−)- or S-(+)-ibuprofen added (Fig. 3B) showed that at the maximal drug concentration applied, i.e., at 43.6 mg/l, ibuprofen inhibited practically all of the degradation of the high molecular weight hyaluronan sample during the whole time course of the experiment. Addition of the drug at a lower level studied, i.e., 31.3, 22.0, 9.6, or 5.1 mg/l, resulted in a decrease of its efficacy in inhibiting HA degradation.

These observations are fully in accord with the law of mass action. Interestingly, however, we demonstrated a slightly higher efficiency of the R-(−)-ibuprofen enantiomer to inhibit/retard the OH- radical-induced hyaluronan degradation than that exerted by the S-(+)-ibuprofen derivative.

DISCUSSION

Design of the study

Practically any one in vitro system used for testing the antioxidative efficacy of a compound/drug commonly contains three basic components: i) an appropriate source generating reactive/oxidative species; ii) the antioxidant whose efficacy has to be tested; and iii) an appropriate marker indicating the course of the reaction (13).

In our study arrangement, hydrogen peroxide (c = 8.0 mmol/l) along with the transition metal ions of copper (c = 0.2 mmol/l) yielded a constant flux of OH radicals (6). Since the presence of both H$_2$O$_2$ and Cu$^{2+}$ was demonstrated in the synovial fluid in inflammatory diseases of the joints (14), the chosen generator of the free radicals may be considered to have a qualitative composition closely related to the given specific pathophysiological situation.
The selection of high molecular weight hyaluronan as a marker of the course of the reaction involving OH radicals has been repeatedly substantiated (4, 6, 15-18). Namely, the hyaluronan macrobiomolecular chain is degraded by the action of $\text{H}_2\text{O}_2 + \text{Cu}^{2+}$, with both agents required for efficient degradation reaction. It has been found that OH radicals produce HA backbone breaks with an efficiency of 21%; i.e., for every 100 OH radicals reacting with the high molecular weight hyaluronan, 21 lead to biopolymer chain breakage (19).

The molecular interpretation of the high molecular weight hyaluronan degradation reaction brought on by OH radicals appears to be evident. On attacking the backbone of the biopolymer (20), the hydroxyl radical breaks up the macromolecular strand (6, 18) thus reducing the molecular weight of the biopolymer.

**Chemistry of the hyaluronan degradation reaction**

In keeping with observations of other authors, under aerobic conditions, sterile aqueous solution containing high molecular weight hyaluronan plus $\text{CuSO}_4$, as well as $\text{NaCl}$ (i.e., $\text{Cu}^{2+}$, ($\text{SO}_4$$^2-$, $\text{Na}^+$, $\text{Cl}^-$), was not found to show appreciable signs of degradation of the biopolymer. Addition of hydrogen peroxide, however, presented an immediate impulse for the drop of $[?]_{rel}$ value of the solution (Fig. 3A and B).

Reaction of $\text{H}_2\text{O}_2$ with transition metal ion ($\text{M}^{n+}$) results in generation of hydroxyl radicals by the following oxidation-reduction reaction:

$$\text{H}_2\text{O}_2 + \text{M}^{n+} \rightarrow \text{OH} + \text{HO}^- + \text{M}^{(n+1)+} \quad (1)$$

*i.e.*, hydrogen peroxide oxidizes the metal ion from the reduced valency state, $\text{M}^{n+}$, to the oxidized valency state of $\text{M}^{(n+1)+}$, with generation of primary hydroxyl radicals (•OH), directly initiating radical degradation of the high molecular weight HA: the OH radical pulls out the H radical from the hyaluronan macromolecule. The resulting (macro)radical ($\text{A}^*$) reacts, under aerobic conditions, with the $\text{O}_2$ molecule, yielding a (secondary) peroxyl (macro)radical ($\text{A}^*$•-O-O•). The interaction of type $\text{A}^*$•-O-O• (macro)radical with the intact HA macromolecule results, due to the H radical transfer, in the production of a high molecular weight hydroperoxide ($\text{A}^*$•-O-O-H) and the $\text{A}^*$ (macro)radical. Generation of the primary/initiation OH radical does however continue after an oxidation-reduction decomposition reaction of the $\text{A}^*$•-O-O-H macromolecule by the $\text{M}^{n+}$ ion.

It is justified to expect such a mechanism, namely the reaction according to equation (1), when the metal ion is e.g., $\text{Fe}^{2+}$ ($\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{•OH} + \text{HO}^- + \text{Fe}^{3+}$, the so-called simple Fenton reaction) or $\text{Cu}^+$, an ion even more reactive than $\text{Fe}^{2+}$ (21). The involvement of $\text{Cu}^{2+}$ however excludes the occurrence of reaction (1) (i.e., an increase of the oxidation degree of copper to more than 2+). Although the system $\text{H}_2\text{O}_2 + \text{Cu}^{2+}$ resulted in degradation of high molecular weight HA to an extent only somewhat lower than achieved with the system $\text{H}_2\text{O}_2 + \text{Fe}^{2+}$, Miyazaki et al. (21) have suggested that it is the presence of impurities, namely the traces of $\text{Cu}^+$, which may act as initiator of $\text{H}_2\text{O}_2$ decomposition, being thus the immediate source of •OH. Al-Assaf et al. (22) have proposed another "source" of $\text{Cu}^+$, namely from the occurrence of the reaction $\text{H}_2\text{O}_2 + 2\text{Cu}^{2+} \rightarrow 2\text{Cu}^+ + \text{O}_2 + 2\text{H}^+$. In order to accept the possibility that this reaction is the source of $\text{Cu}^+$ generation, hydrogen peroxide would have to act as a reducing agent and become oxidized with the production of molecular oxygen and hydrogen ions and thus the solution would be acidified. In the light of our experimental experience, however, decomposition of $\text{H}_2\text{O}_2$ by $\text{Cu}^{2+}$ ions can not be considered a plausible process.
At present, the most probable interpretation appears to be primarily the reaction of polysaccharide molecules with Cu$^{2+}$ ions as the immediate source of Cu$^{+}$ in our system. In this case HA acts as a reducing agent and by electron donation it reduces Cu$^{2+}$ to Cu$^{+}$. (Evidence of sugar in urine by reduction of Cu$^{2+}$ down to Cu$^{0}$ is one of the "classic" determination reactions of sugar in urine of diabetic patients.) After addition of H$_2$O$_2$, the generated ion Cu$^{+}$ yields *OH, according to reaction (1), followed by degradation of high molecular weight HA.

**Stereochemistry of the hyaluronan degradation reaction**

Under the given experimental pH conditions, the HA macromolecule is highly ionized. Its apparent pK$_a$ value reported (for the D-glucuronic acids) = 3.12 (23).

In aqueous solutions, owing to the intermolecular interactions involved, the relatively stiff HA polyanionic chains associate (aggregate). As a result of this process, the macromolecules form a specific, stable, higher-order structure (24). The anions of the D-glucuronic acids, the structural elements of the HA macromolecule, naturally form salts with the (counter)cations of Cu$^{2+}$. Moreover, as reported formerly (25), hyaluronic acid binds reversibly cupric ions (the binding constant = 3.0 x 10$^{3}$ l/mol (26)). HA plus Cu$^{2+}$ form namely a "complex"/"coordinate" compound in which the cupric cation is fixed by two carboxyl anions originating from either one or two HA chains. (The authors proposing such coordinated structures stated also that while the Cu$^{2+}$ bound "intramolecularly" resulted in forming a water-soluble associate/complex, in the case of "intermolecular cross-links" the aggregate formed was water insoluble (25).)

Under the experimental conditions used, ibuprofen molecules (pK$_a$ = 4.4 (27)) bear a negative charge. The upper drug concentration set = 43.6 mg/l (0.2 mmol/l) inhibited practically all HA degradation (Fig. 3A). This action may originate directly from the complete "fixation"/"trapping" of the Cu$^{2+}$ ions, since the two constituents (i.e., ibuprofen anions and cupric cations) at the highest drug concentration set form with Cu$^{2+}$ an equimolar system [0.2 mmol/l:0.2 mmol/l]. At lower drug levels, the concentration of the "free"/"untrapped" Cu$^{2+}$ predominated and at the lowest drug concentration used (5.1 mg/l i.e., 0.02 mmol/l), there was a high enough free cupric ion level which, with H$_2$O$_2$ present, degraded broadly the HA probe (Fig. 3A).

An intriguing finding, which could be better elucidated on being investigated at the molecular level, is the very nature of the reaction. The reaction displays a certain induction period (of about 1 h), which represents a retardation of the HA degradation, to be followed by a very fast decomposition process. This type of (induced) degradation reaction has been reported by other authors as well, suggesting that the HA chains are broken down as a consequence of the two processes, one very fast and a slower (thermal) process (20). Moreover, they maintained that the fast process was more likely to be due to ("undetected") radicals formed "at other sites on the HA molecule" (a site-specific damage of HA by OH radicals), and that the slow (secondary) process arose either from more stable radicals or from semi-stable initial products such as hydroperoxides.

**Stereoselectivity in the inhibition of HA degradation**

In the light of the stereochemical nature of the process, however, the most stimulating finding appears to be the detection of differences in the kinetics of HA degradation in a system containing one single ibuprofen enantiomer (Fig. 3B). At this stage of the research it is conceivable that the HA molecule produces with copper ions and with the R()-ibuprofen molecule a certain higher-order structure (complex, associate), which is more stable/resistant to decomposition induced by the addition of H$_2$O$_2$. On the other hand, the complex HA + copper ion(s) + S-()+-ibuprofen is either less resistant to the
attack of *OH, or it may more readily exchange electrons, thus allowing a stronger flux of primary (initiating) OH radicals, or of secondary peroxy radicals.

Our experimental results indicate that a more thorough investigation of the problem would be beneficial. This appears to be justified also by the fact that the majority of NSAIDs used at present are actually a mixture of two isomers/enantiomers. Such investigations, supplemented by detailed studies of (positive) effects of utilizing complexes of copper (ions) in the treatment of inflammatory rheumatic diseases, would provide insight into the effect of the given drugs and their mechanisms of action at molecular level.

**Consequences for in vivo situations**

In the synovial fluid (SF) of the healthy human population hyaluronan concentration equals 2-3 g/l, i.e., 0.2-0.3-% (w/v) (28). In arthritic diseases, the SF volume is abundant (29, 30), the concentration of hyaluronan is decreased and the HA molecular weight is reduced (31). The loss of SF viscosity within the arthritic joint is accompanied by the infiltration of large numbers of polymorphonuclear leukocytes into the joint space, which release reactive oxygen species during the respiratory burst (28, 32-34). The appearing pain is predominantly caused by the reduced ability of the compromised SF to lessen the (mechanical) friction of the bone endings forming the joint, yet inflammation of the joint tissue is also involved. The pain is dulled by NSAIDs, e.g., ibuprofen.

The simultaneous disposition of the two ibuprofen enantiomers in synovial fluid and plasma was monitored in eight patients with arthritis (35). In SF the S-(+)-enantiomer concentrations exceeded those of the R-()-enantiomer in all patients at all times, with the ratio of S- to R-levels being 2.1 ± 0.3. Synovial fluid concentrations of R-()-ibuprofen exceeded those in plasma starting at a mean of 5.4 ± 0.3 h after RS-(±)-drug racemate administration. Similarly, SF concentrations of S-(+) ibuprofen exceeded those in plasma starting at a mean of 5.5 ± 0.6 h after drug ingestion. The levels of the individual enantiomers in SF at these times (5-6 h) ranged approximately between 3-5 mg/l for the R-()- and between 6-14 mg/l for the S-(+) enantiomer. It was at these drug concentrations when a higher OH-radical degradation inhibitory action of the R-()-isomer was indicative (Fig. 3B). It may thus be stated that although the S-(+) ibuprofen enantiomer blocks the pain with a significantly greater efficiency than does the R-()-ibuprofen, the latter is the one whose free radical scavenging action, or its contribution to this action is predominant.

Finally, the well-documented treatment with the RS-(±)-ibuprofen racemate for over 30 years speaks in favor of its use and does not appear inevitable to substitute it by the single S-(+) ibuprofen derivative. Nevertheless, clinical studies should be broadened to gain insight into the fate of both HA and ibuprofen enantiomers during an arthritic situation by monitoring all system components, i.e., the two enantiomers of the parent drug, their metabolites, and the possible changes in HA molecular weights. Namely, hyaluronan attacked in vitro by OH radicals yielded several intermediates and end-products (18), which were also found in increased concentrations in the synovial fluid (and serum) of rheumatic patients (36). The synovial fluid is thus the relevant compartment whose monitoring could substantially improve the understanding of both the duration of the drug action and of its fate in this (arthritic) micro-environment.

**CONCLUSIONS**

Under the experimental conditions used, the OH-radical-induced HA degradation was strictly time-dependent with prolonged exposure times resulting in a more pronounced [%] rel. decrease. When ibuprofen was present in the system, its inhibitory effect was clearly evidenced. The kinetics of the HA degradation reaction was dependent on the drug level set in the system. Of the two ibuprofen enantiomers, the R-()-drug isomer demonstrated a higher protective effect against the OH-radical degradation of hyaluronan.
ACKNOWLEDGMENTS

The grants VEGA No. 1047, 5022 and 6027 from the Grant Agency for Sciences, Bratislava, Slovak Republic are gratefully acknowledged.

References


3. Óoltés, L., Mislovi...ová, D., Sébille, B. Insight into the distribution of molecular weights and higher-order structure of hyaluronans and some \( \beta-(1 \rightarrow 3) \) glucans by size exclusion chromatography. Biomed Chromatogr 1996, 10: 53-9.


---

**Address for correspondence:** Ladislav Óoltés, Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, SK-842 16 Bratislava, Slovak Republic. E-mail: exfasolt@savba.sk

*Dedicated to the architect of the "Hyaluronan-a-Polis", Dr. Endre A. Balázs.*

Methods and Findings in Experimental and Clinical Pharmacology Vol. 23, No. 2, 2001, pp. 85-87
http://www.prous.com

All rights reserved. Legal Information.