Synegistic interaction between TS-polysaccharide and hyaluronic acid: implications in the formulation of lacrimal substitutes

Giacomo Di Colo[a], Ylenia Zambito[a], Sara Ottino[a], Marco Sansò[b]*

a - Dipartimento di Chimica Bioorganica e Biofarmaci-Università di Pisa,V. Bonanno 33, I-56126 Pisa (Italy)
b - Farmigea S.p.A.,V. G.B. Oliva 6/8, I-56121 Pisa (Italy)
* - e-mail: zambito@farm.unipi.it

Introduction: Recently two polysaccharides of natural origin, namely, tamarind seed polysaccharide (TSP) and hyaluronic acid (HA), have been demonstrated by both in vitro and in vivo tests to be mucoadhesive at the ocular surface of rabbits, thereby stabilizing the tear film and prolonging the retention of ophthalmic drugs of different chemical nature, such as ketotifen fumarate (KF) and diclofenac sodium (DS), in the precorneal area [1]. A possible interpolimer TSP-HA non-covalent interaction may generate an excipient for eyedrops, comprised of a mixture of these polysaccharides, having synergistically improved properties over those of the single polymers. The present study was aimed at ascertaining the possibility of TSP and HA undergoing interpolymer interactions, and finding out the composition of the TSP/HA mixture maximizing the interaction strength.

Methods

For comparing the polysaccharides for their adhesiveness to the ocular surface ophthalmic drops containing FITC-labeled TSP-HA mixtures at the total concentration of 0.5% w/v in isotonic phosphate buffered (0.0375 M) saline pH 7.4 (PBS) were prepared. The polymers were in the following wt ratios: TSP-HA 1:4; TSP-HA 2:3; TSP-HA 3:2; TSP-HA 4:1. Medicated ophthalmic drops were prepared, containing 0.7 mg/ml KF or 1 mg/ml DS in the solutions. In a dispersion of 15% w/v porcine gastric mucin and the polymer mixture, having viscosity η, the viscosity component due to mucin-polymer interaction, η_{mp}, is calculated as [2]:

$$\eta_{mp}=\eta-\eta_m-\eta_p$$

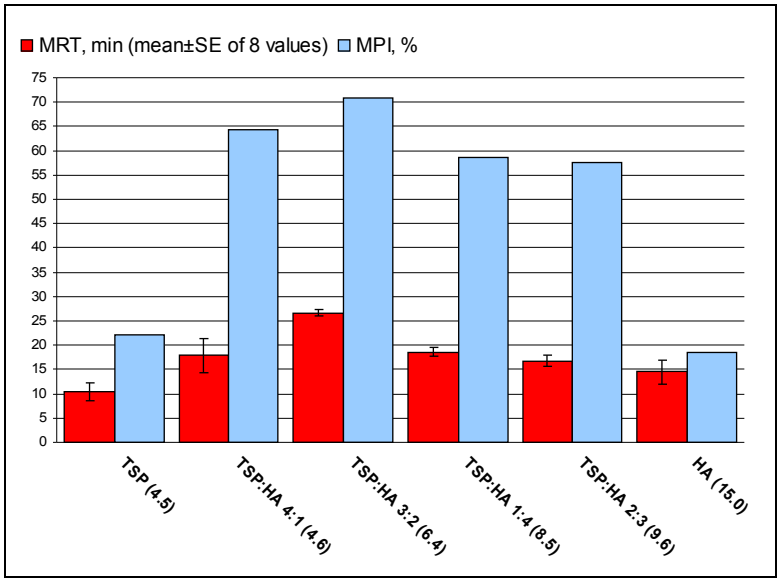
where η_m and η_p are the viscosities of mucin and polymer, respectively. A mucin-polysaccharide interactivity parameter (MPI) is defined as $MPI=\eta_{mp}/\eta$ and used to compare polymer mucoadhesiveness. Elimination kinetics of polysaccharides, or drugs, from rabbit tear fluid were determined by analyzing 1.0-μl of it, at intervals, for the polymer, by a fluorimeter, or the drug, by HPLC. For each elimination curve an MRT value was calculated as AUMC/AUC. The maximum residence time of each drug at quantifiable concentrations in tear fluid (RT_{max}) corresponds to the last point of the elimination curve (minimum quantifiable concentration, 1.1 μg/ml for KF, 1.5 μg/ml for DS). Experiments on dugs permeation across excised rabbit cornea were carried out [3].

Results and Discussion

FIGURE 1 - Comparison between rank orders of mean polysaccharide residence time in tear fluid of rabbits (MRT) and mucin-polysaccharide interactivity (MPI). Polymer mixtures are ordered according to increasing viscosity, indicated within brackets as mPas.

For the mixtures the MPI values are greater than for the unmixed polysaccharides, in particular, the importance of the interactive component is uppermost for the TSP-HA 3:2 mixture. A similar trend is shown by the MRT values. The mean MRT values for the

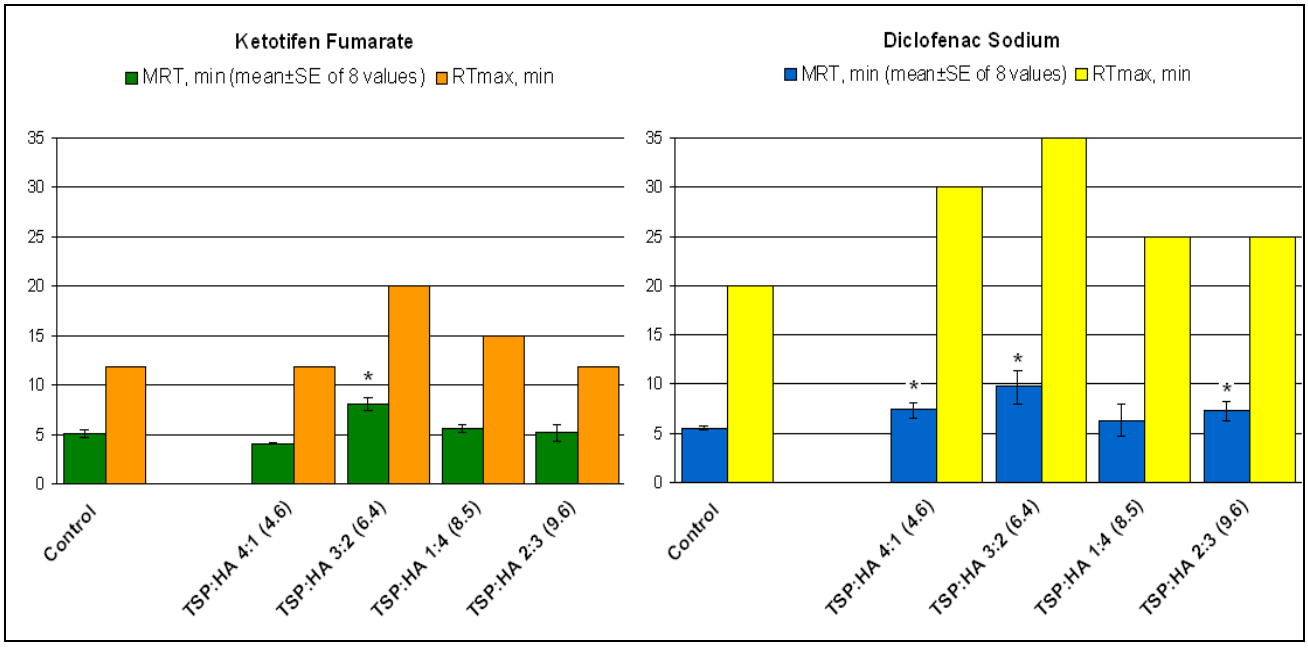
polysaccharide mixtures being higher than those for the unmixed polymers cannot be ascribed to higher viscosity coefficients for the former. Indeed the viscosity coefficient of unmixed HA is substantially higher than the respective values for the mixtures while the viscosity coefficient of unmixed TSP is equal to that of TSP-HA 4:1 spite of neatly lower MPI and MRT values. Then the MPI and MRT values agree in indicating a mucoadhesivity



in

of the mixed polysaccharides stronger than that of the unmixed ones, and hence, a synergistically enhanced mucoadhesivity of these polymers.

FIGURE 2 - Effects of polysaccharides on mean residence time (MRT) and maximum residence time at quantifiable concentrations (RT_{max}) of drugs in tear fluid of rabbits. Polymers are ordered according to increasing viscosity, indicated within brackets as mPas. Data marked by * are statistically different from control (P<0.05)



It appears that the TSP-HA 3:2 mixture has more ability than the others to retain KT and DS on the ocular surface, in fact, the MRT and RT_{max} values for this mixture is the only one significantly higher than the control. DS is chemically quite different from KF, e. g., the former is an acid, the latter a base. Yet, the data on DS residence in precorneal area show some similarities to the corresponding data for KT. The present data altogether suggest that the vehicle, if mucoadhesive, exerts a significant influence on the drug residence time in the precorneal area, irrespective of the chemical nature of the drug.

The P_{app} values listed in the Table show the absence of any statistically significant modification of DS permeability across the cornea by TSP-HA 3:2 with respect to the relevant control. This observation suggests that the interaction of this polysaccharide

mixture with the mucins of the corneal epithelium failed to promote any significant polysaccharide interaction with epithelial cells resulting in an enhancement of epithelial permeability to DS. With KT the TSP-HA 3:2 effect on P_{app} was statistically significant, only it resulted in a 26% decrease rather than an increase. To explain this finding the hypothesis is here advanced that the adhesion of polysaccharides to the membrane-associated mucins, rather than facilitating a permeabilizing action of polymers on the epithelium, might somewhat increase the epithelium barrier properties. This effect had a different strength with DS and KT because of the different chemical nature of these molecules.

TABLE 1 Data on KT or DS permeation across excised rabbit cornea from GBR containing 0.7 mg/ml KT or 1 mg/ml DS and TSP-HA 3:2. Means ± SD (n = 6).

Donor	Flux (μg cm^{-2} h^{-1})	f_F [a]	P_{app} [b] 10^5(cm s^{-1})
KT, control	62.06±4.19	-	2.46±0.17
KT, TSP-HA(3:2)	42.80±2.36*	0.94	1.81±0.10*
DS, control	102.45±8.12	-	2.85±0.23
DS, TSP-HA(3:2)	82.63±8.33*	0.90	2.55±0.26

[a] Drug fraction free from binding to polymers. [b] Apparent permeability.
* Significantly different from the respective control ($P < 0.05$).

Conclusions

In virtue of its mucoadhesivity the TSP-HA 3:2 mixture showed the uppermost ability to resist elimination from tear fluid and stabilize the tear film thereby prolonging the residence of KT and DS in the precorneal area. This enhanced retention points to a potential of KT or DS eyedrops, containing the TSP-HA 3:2 mixture as excipient, to decrease the frequency of instillations. The permeation experiments have shown that the above polysaccharide mixture is unable to permeabilize the corneal epithelium, then mucoadhesivity is the property of TSP-HA 3:2 mainly responsible for its effect to enhance either the extra- or intra-ocular bioavailability by prolonging drug contact with its action/absorption site.

References

[1] Di Colo G., Zambito Y., Zaino C., Sansò M., Drug Dev. Ind. Pharm., 2009, 85, 941.
[2] Hassan E.E. & Gallo J.M., Pharm. Res., 1990, 7, 491.
[3] Zambito Y., Zaino C., Burchielli S., Carelli V., Serafini M.F., Di Colo G., J. Drug Del. Sci. Technol., 2007, 17, 19.

Farmigea SpA - Via G.B. Oliva 8, Pisa (PI) – Italy - www.farmigea.it