Role of high concentrations of mannitol on the stability of hyaluronan in an oxidative stress model induced by xanthine/xanthine oxidase

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Background

Osteoarthritis (OA) is a degenerative joint disease associated with harmful action of reactive oxygen species (ROS).

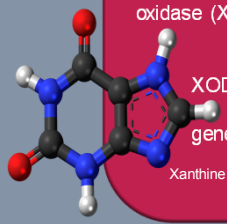
ROS are involved in the degradation of both aggrecan and high-molecular-weight hyaluronan (HMW-HA). The later plays a key-role in the joint lubrication and the visco-elastic and shock absorbing properties of the synovial fluid (SF).

Viscosupplementation consists in injecting intra-articularly exogenous HMW-HA to restore the SF rheological properties, that are dramatically decreased in OA.

However the injected HA is also rapidly degraded by ROS, decreasing its effectiveness and duration of action.

Objective

To evaluate the ability of Mannitol , a powerful oxygen free radical scavenger, to reduce exogenous HMW-HA degradation using a model of oxidative stress induced by xanthine (X) + xanthine oxidase (XOD).



XOD is a flavoprotein that catalyzes oxidation of hypoxanthine to xanthine and then to uric acid generating high levels of superoxide anion.

Xanthine

Methods

Hyaluronan (MW# 0.8mDa) was submitted to an oxidative stress generated by the addition of X + XOD.

Then solution of the same HA + 35g/L of Mannitol in PBS buffer was studied.

Different enzyme concentrations (XOD 109 mUI/mL and 218 mUI/mL) were used and the HA properties were studied after 24 hours of contact at ambient temperature.

Changes of the viscosity of the solution were assessed by rheometry (using a rheometer at 25° C with a cone and plate geometry, steady-state viscosity was determined in Pa.s, as a function of the shear rate).

Rheometer : AR 1000 from TA instruments

HA MW was determined by **steric exclusion chromatography** before and after oxidative stress.

Results

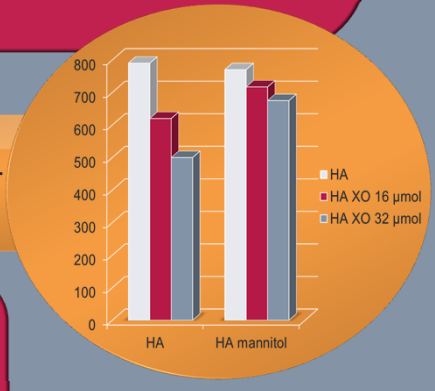
The presence of X/XOD degraded HA in the conditions tested :

- ► HA viscosity decreased as a function of XOD concentration,
- ► HA MW decreased dramatically by 36.6%.

	Initial	+16 microL enz.	+32 microL enz.
HA	798 000 / 776 000	625 600 / 617 300	503 800 / 498 900
HA / Mannitol	781 200 / 756 600	762 200 / 673 300	674 000 / 680 300

On the opposite, in presence of high concentration of Mannitol :

- ► HA viscosity was stable,
- ► HA MW decreased only slightly (-11.9%).



Variation of the HA molecular weight (kDa) after oxidative stress induced by xanthine/xanthine oxidase at various concentrations

Conclusion

High concentrations (3.5%) of **mannitol** protect HA from ROS-mediated degradation.

These in vitro data suggest **that mannitol may increase the intra-articular residence time of HA** and consequently may improve clinical efficacy of viscosupplementation.