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Preclinical pharmacokinetics of radiolabelled hyaluronan

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Abstract:
Background: Hyaluronan (HYA) is a high molecular weight glucosaminoglycan with a great perspective for medical applications. Because HYA is widespread in the body, it is difficult to determine the fate of exogenously administered HYA.
Methods: In this study, HYA of different molecular weights (0.1–1 MDa) was labelled with ^{99m}Tc, and the distribution profiles were determined after administrating the HYA to rats.
Results: After the intravenous administration of ^{99m}Tc-HYA, a rapid decrease in the radioactivity of blood samples was observed, presumably because of ^{99m}Tc-HYA uptake by the liver; only minimal signs of liver radioactivity washout were detected. After the oral administration of ^{99m}Tc-HYA, no significant absorption to the central compartment was found. A preliminary study using ^{14}C-HYA exhibited a different distribution profile than ^{99m}Tc-HYA because of the different administered dose and the fate of the degradation products. Even with ^{14}C-HYA, only traces of radioactivity were absorbed after oral administration.
Conclusion: This paper provides quantitative information regarding the distribution parameters of radiolabelled HYA in preclinical experiments.

Key words:
hyaluronan, ^{14}C, ^{99m}Tc, radiolabelling, biodistribution, pharmacokinetics

Introduction

Hyaluronan (HYA) is a nonsulfated macromolecular glucosaminoglycan found ubiquitously in nearly all vertebrate tissues, mainly in the extracellular matrix. HYA is composed of repetitive disaccharide units of N-acetylglucosamine and D-glucuronic acid, and the molecular weight of HYA is typically over 1 MDa. The biological effects of HYA are complex; it participates in various physiological processes, including cell-matrix interactions, cell proliferation, cell motility, tissue fluid homeostasis, wound repair, inflammation, atherosclerosis, angiogenesis, tumorigenesis and embryonic tissue development. Thus, HYA-composed materials may mimic those conditions favorable for tissue growth and regeneration [5, 10, 11, 13]. The specific receptors for HYA and its fragments are present in all cells [2], and the biological activities of HYA are dependent on the molecular weight.

Because of its biocompatibility, nonimmunogenicity and unique viscoelastic and lubricating properties, HYA is used for a variety of medical device applications. For example, HYA is used for wound healing,

eye surgery, arthritis, tendon repair, nerve guides, controlled drug release matrices, cosmetic applications, and dietary supplements for animals and humans. In addition, HYA is employed as a promising drug delivery system for drugs that target tumor cells because the HYA receptors, CD44 and RHAMM, are overexpressed in a variety of tumors [9]. HYA is predominantly administered intravenously or topically, and it is believed that orally administered HYA is poorly absorbed and efficient. Unfortunately, information concerning the pharmacokinetics of exogenous HYA is lacking because exogenously administered HYA cannot be directly measured by any analytical method; endogenous HYA is found everywhere in the body. For this reason, labelling of HYA with chromophores, luminofores or radiotracers is usually employed in pharmacokinetic studies of HYA. HYA can be directly radiolabelled with a selected radiotracer. Moreover, a bifunctional labelling approach using radiometals and radioiodination *via* a reactive aromatic prosthetic group has been employed in several studies. Radiolabelling enables a simple and detailed analysis of the consequences of exogenously administered HYA in the body. However, these methods have some limitations. Radiolabelled species may exhibit different biological properties than the parent compound because of the presence of the unnatural groups introduced into the HYA structure. Moreover, the parent molecule may degrade soon after administration, so a mixture of HYA and radiolabelled degradation products may be observed. Isotope labelling of HYA with ^{3}H and ^{14}C by biotechnological synthesis has been proposed to overcome these limitations. However, this labelling procedure is very expensive, is time consuming, and requires a special apparatus. In addition, the low specific activity of the product limits the detection of HYA in the body. Therefore, it is difficult to determine low levels of HYA and its radiolabelled fragments in biological samples.

The purpose of this study was to analyze the distribution profiles and elimination pathways of ^{99m}Tc-HYA of different molecular weights after intravenous and oral administration to rats. The results were compared with those obtained for ^{14}C-labelled HYA administration in order to determine the effect of the radiolabel on the pharmacokinetic characteristics of HYA. Employing different radiolabelling strategies may help elucidate the HYA distribution profile throughout the body and aid in the interpretation of the results.

Materials and Methods

HYA

HYA of different molecular weights was prepared and characterized by CPN Ltd. (Dolni Dobrouc, Czech Republic). All other chemicals were of analytical grade.

Chemicals and radiotracers

^{99m}Tc-pertechnetate was obtained by elution of the ^{99}Mo-^{99m}Tc generator DRYTEC (GE Healthcare Ltd., UK).

Glucose, D-[^{14}C(U)] (13.3 GBq/mmol, 37 MBq/ml), liquid scintillation cocktail (Ultima Gold^{TM}) and aqueous tissue solubilizer (Solvable^{TM}) were purchased from PerkinElmer Life and Analytical Sciences (USA).

Artificial gastric juice was prepared for stability studies by dissolving 2 g of NaCl (BioXtra, Sigma) and 3.2 g of pepsin (from porcine gastric mucosa, Sigma) in water. After dissolution, 80 ml of HCl (1 mol/l) was added, and the volume of the solution was brought to 1 l with demineralized water.

Radiolabelling and quality control

For HYA radiolabelling with ^{99m}Tc, a direct labelling method was employed. Nine milligrams of HYA (100 kDa, 0.5 MDa or 1 MDa) was mixed with 900 µl of distilled water (Millipore Quality) in 2 ml Eppendorf tubes on an electromagnetic stirrer overnight at room temperature. Then, 30 µl of 10^{-3} M aqueous calcium glucoheptonate (Sigma), 1 ml of ^{99m}Tc-pertechnetate (A 10 mCi) in saline and 100 µl of 4×10^{-4} M $SnCl_2$ in 1 M HCl were added. After incubation at 90°C for 50 min, radiolabelled HYA was purified by gel permeation chromatography (GPC) on a Sephadex G-50 column. Separation was performed on glass 30 × 1 cm column with Sephadex G-50 as the solid phase and demineralized water as the mobile phase. For the biological experiments, the concentration of HYA labelled with ^{99m}Tc was 0.6 mg/ml (D = 0.12 mg per animal).

The stability of ^{99m}Tc-HYA was determined in saline (pH 7.5, 25°C), fresh rat plasma (37°C) and artificial gastric juice (pH 1.7, 37°C [3]). A solution of ^{99m}Tc-HYA was mixed with the above mentioned media at a 1:1 (v/v) ratio, and the mixture was analyzed for radiochemical purity at selected time points after mixing.