ABSTRACT

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Title of Doctoral Thesis **Analysis of radiolabeled hyaluronic acid and its metabolites in biological material**

The presented dissertation thesis deals with an optimization process of radiolabeled techniques and the following analysis of radioactive HA in biological material.

Theoretical part include basic information about physical, chemical and biological properties of HA, and its function and degradation processes in vitro and in vivo. Furthermore, there are described analytical methods for the determination of HA, and also some of the most employed radiolabeled techniques of HA and its derivatives. Last but not least, there are summarized all the latest knowledge about biodistribution and pharmacokinetics of HA after intravenous, or topical administration, etc. In addition, we can also find there the most often medical and cosmetic application of HA.

Radiolabeled techniques using four different radionuclides (111In, 125I, 14C and 99mTc) were developed: chemical synthesis, biosynthesis, and direct labeling method. Consequently, purification process of radiolabeled HA using separation on sephadex G-50 or membrane filtration was optimized. The purpose of purification process was to obtain the final product (radioactive HA) with a sufficient specific activity, and high radiochemical purity. Radiochemical purity of radioactive HA was determined using HPLC with highly sensitive radiometric detection.

As the next issue, the kinetic stability of radioactive HA with different molecular weight was studied. Stability studies are required before radiolabeled HA in biological experiments is employed, especially if chelating moiety in HA molecule is present. Therefore, the stability of radioactive HA was assessed in three different mediums: acetate buffer (pH 7.5) at 25 ºC, artificial gastric juice (pH 1.7) at 37 ºC, and fresh rat plasma at 37 ºC. Finally, all of the radiolabeled techniques were compared. The evaluated parameters were: laboriousness of the method, costs, kinetic stability, the final yield of labeling and purification process, specific activity and radiochemical purity of radiolabeled HA.

The final subject we deal with, was the pharmacokinetics of radiolabeled HA (111In-DTPA-HA, 125I-Tm(Trz)-HA, 14C-HA a 99mTc-HA) after intravenous and oral administration to male Wistar rats. All of the biological experiments were accomplished by the workers of the Section of Radiopharmacy, Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Pharmacy in Hradec Králové. The aim of these experiments was to find out the presence or absence of activity in joints after oral administration. In conclusion, the results were compared with the world′s technical journal literature.

All acquired data were the part of project which was supported by companies CPN, s.r.o. and Contipro C, a.s., which are subsidiaries of holding Contipro Group, Dolní Dobrouč, Czech Republic.