Heparin from Bovine Intestinal Mucosa: Glycans with Multiple Sulfation Patterns and Anticoagulant Effects

Ana M.F. Tovar, Nina V.M. Capillé, Gustavo R. C. Santos, Bruno C. Vairo, Stephan-Nicollas M. C. G. Oliveira, Roberto J. C. Fonseca and Paulo A.S. Mourão*1

1 Laboratório de Tecido Conjuntivo, Hospital Universitário Clementino Fraga Filho and Programa de Glicobiologia, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Caixa Postal 68041, Rio de Janeiro, RJ, 21941-590, Brazil

Pharmaceutical grade heparins from porcine intestine and bovine lung consist mainly of repeating tri-sulfated units, of the disaccharide $4^{-}\alpha$-IdoA2S-1$\rightarrow$$4^{-}\alpha$-GlcNS6S-1$\rightarrow$. Heparin preparations from bovine intestine, in contrast, are more heterogeneous. NMR and disaccharide analysis after heparinase digestions show that heparin from bovine intestine contains $\alpha$-glucosamine with significant substitutive variations: 64% are 6-O-sulfated and N-sulfated, as in porcine intestinal heparin while 36% are 6-desulfated. Desulfated $\alpha$-iduronic acid units are in slight lower proportions in bovine than in porcine heparin. NMR data also indicate N-, 3- and 6-trisulfated $\alpha$-glucosamine (lower proportions) and $\alpha$-GlcNS-1$\rightarrow$$4^{-}\beta$-GlcA and $\alpha$-IdoA2S-1$\rightarrow$$4^{-}\alpha$-GlcNAc (higher amounts) in bovine than in porcine heparin. Porcine and bovine heparins can be fractionated by anion exchange chromatography into three fractions containing different substitutions on the $\alpha$-glucosamine units. Each individual fraction shows close disaccharide composition and anticoagulant activity, regardless of their origin (bovine or porcine intestine). However, these two heparins differ markedly in the proportions of the three fractions. Interestingly, fractions with the typical heparin disaccharides of porcine intestine are present in bovine intestinal heparin. These fractions contain high in vitro anticoagulant activity, reduced antithrombotic effect and high bleeding tendency. These observations indicate that the prediction of haemostatic effects of heparin preparations cannot rely exclusively on structural analysis and anticoagulant assays in vitro. Minor structural components may account for variations on in vivo effects. In conclusion, we suggest that pharmaceutical grade bovine intestinal heparin, even after purification procedures, is not an equivalent drug to porcine intestinal heparin.

Key words: Thrombosis, glycosaminoglycan, bleeding tendency.
Supported by: CNPq, FAPERJ and CAPES.