Heparin Clearance by Liver Scavenger Receptors

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The liver is one of the primary scavenger organs for the blood and has been the ire of pharmaceutical companies that seek to keep drugs from getting metabolized by this organ. There are four main cellular populations of the liver: hepatocytes (HCs), endothelial cells (SECs), Kupffer cells (KCs), and stellate cells (HSCs). HCs are nearly entirely metabolic centric in that they regulate blood sugar by producing/catabolizing glycogen, produce urea and albumin, among many other functions. HSCs store vitamin A and are the immune regulatory cells of the liver. KCs are highly phagocytic and engulf apoptotic bodies, dead red blood cells and keep potential pathogens in check. SECs contain a fenestrated cell body which are clusters of holes or sieve plates that act as filters for the HCs and HSCs (Figure 1). One of the first discovered macromolecules of the body to be regulated by these cells is hyaluronic acid (HA) which is cleared from blood by receptor-mediated endocytosis. This receptor activity has been detected since the early 1980s when Frasier and co-workers injected rabbits with radiolabeled hyaluronic acid [1]. During the remainder of the decade, hyaluronic acid turnover from solid tissue extracellular matrix to lymph to blood was understood in terms of kinetic action [2-4]. However, the mechanism was not well understood as the receptors and syntheses for HA were not yet identified.

In the mid-1990s, Dr. Paul Weigel and coworkers discovered the receptor for internalizing HA by purifying rat LSECs using a modified protocol [5], first published by P.O. Seglen [6], followed by the 125I-HA ligand blot technique to identify bands from a set of proteins separated by SDS-PAGE that bound HA with high affinity. The protein was enriched and monoclonal antibodies were produced from protein bands cut from acrylamide gels. With these antibodies, the receptor was more thoroughly defined and was first cloned from rat liver [7-10]. The cloned rat receptor, called 175-HARE due to its molecular mass on 5% SDS-PAGE of 175 kDa, was characterized and found to bind HA and many of the chondroitin sulfates that directly competed with HA for binding [11]. The HARE receptor was found not only in liver endothelium but also in the sinusoids of spleen and lymph node as a complex of proteins that were bound by disulfide bonds [9,12]. What also became clear is that both isoforms of HARE were expressed in all tissue analyzed and that the smaller isoform seemed to be as abundant if not more so in tissue. In recombinant cell lines, the larger isoform is 4-5 times more abundant than the smaller isoform and the mechanism for production of the smaller isoform is still not known. From these studies, it was becoming clear that this scavenger receptor had multiple ligands for ECM material which may have a dynamic turnover that was not fully appreciated at the time.

In 2001, the human HARE receptor was cloned in its entirety by a different group [13] and then further characterized independently by Paul Weigel’s laboratory in 2004 and 2007 [14,15]. Due to its structural and organizational homology to Stabilin-1, the HARE receptor was formally named Stabilin-2. The human receptor proved to be one large receptor, that when expressed in recombinant cells such as HEK293 human kidney cells, was expressed as two isoforms of 315-kDa and 190-kDa as analyzed by SDS-PAGE. The 190-HARE is the C-terminal membrane-bound part of the full-length receptor (Figure 2). The full-length rat receptor has never been cloned, although the 175-HARE, like the 190-HARE, is an active receptor. Like the rat receptor, human Stabilin-2 also bound HA via the LINK (X-LINK) domain and competed with chondroitin sulfates (CS) A-E, all with different affinities [15]. With the use of direct labeling methods, it was discovered that human Stabilin-2 bound to heparin, a glycosaminoglycan similar to the chondroitin sulfates [16]. This was a significant discovery since the clearance of heparin from blood was not well-characterized and was relegated to the reticuloendothelial system for general clearance. The mechanism of heparin binding eluded previous ligand screens.

Figure 1: Scanning electron micrograph of a rat liver sinusoid revealing a cross section of the SEC with hepatocyte frimbriae coming through the sieve plates. Each hole of the sieve plate is 100-150 nm in diameter. Magnification = 10,000x.

Figure 2: Domain organizations of Stabilin-1 and Stabilin-2. Both receptors are of similar size and are 41% identical and 56% similar. Recombinant Stabilin-1 is expressed as a tight doublet in the 320 kDa range and Stabilin-2 is expressed with near equal amounts of 315 and 190 kDa isoforms.

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due to the fact that heparin does not compete with 125I-HA on ligand
blots or ELISA-like assays, has high background due to the polymer’s
electronegativity, and does not bind to the LINK domain of Stabilin-2
[17]. The exact binding site for heparin is still not known, but that
site also binds with other heparinoid polymers such as chondroitin
sulfate B/dermatan sulfate and chondroitin sulfate E. Curiously, CS-E
(4.6 disulfate) and CS-D (2.6 disulfate) are very similar except for the
position of one sulfate group, yet have entirely different binding profiles
against Stabilin-2. The affinity for heparin was found to be in the 40-
60 nM range, higher than the 300 nM threshold needed for clearance
activity from human blood [18].

There has been recent interest in the clearance of heparin due to the
development of synthetic heparin. Heparin is a polydisperse polysaccharide
made up of the disaccharides glucuronic/iduronic acid and N-sulfated glucosamine. Both sugars are sulfated and the degree of
sulfation and ratio of glucuronic acid and iduronic acid determine
whether the polysaccharide is heparin or heparan sulfate [19]. Medical
forms of heparin come in three different grades; unfractionated heparin (UFH, polysaccharides from 3,000-30,000 Da), low-molecular
weight heparin (LMWH, polysaccharides from 2,000 to 8,000 Da) and
Fondaparinux (chemically synthetic pentasaccharide). Unlike current
medical grade heparin, which is isolated from pig intestine, chemo-
enzymatically synthesized heparin from cloned heparin/heparan sulfateiosynthetic enzymes [20-22] is a potentially cost-effective method for
obtaining heparin mono-disperse polysaccharides with homogenous
modifications. Work in my laboratory, which studies heparin
interactions in SECs and specifically with the Stabilin-2 receptor, shed
some light on what forms of chemo-enzymatically synthesized heparin
is required for clearance. From our cell culture work, we found that
both Stabilin-1 and Stabilin-2 have the same binding characteristics for
heparins and that the polysaccharides had to be at least 10 sugars long
with a 3-O sulfated GlcNS to be efficiently internalized within the cell
[23]. Our future work remains to see how freshly cultured SECs bind and internalize heparins, how the chemo-enzymatically synthesized heparins are cleared from blood, what structural modifications are
required for physiological clearance vs. anti-coagulation, and the interplay between hepatic and renal clearance for customized mono-
disperse heparins.

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