Role of liver sinusoidal endothelial cells (LSECs) in the systemic catabolism of connective tissue macromolecules

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In studies on connective tissue macromolecules (CTM) biosynthesis have been generally more explored than degradation. Yet, few scientists would deny that the degradative and synthetic arms are equally important. Understanding the catabolism of these substances is a matter of understanding where degradation takes place. In fact, the catabolism of these molecules often takes place at a distance from the cellular site where they were produced. For this reason it would be necessary to ask not only "how" degradation of CTM is brought about, it is similarly important to ask "where". Yet, few scientists have asked that question. In fact, it is generally taken for granted that degradation of CTM takes place locally in the tissue, close to where it was synthetized. This is only partially correct: the local degradation of CTMs includes in general only one or a few proteolytic clips that release the CTM form the matrix. The question that is generally very rarely asked is: What is the catabolic fate of these large proteolytic fragments of CTM? We have studied this, both through own experiments and via the literature over the last 3 decades, and found the following sequence of events: i) relatively large CTM fragments are released from the matrix by proteolytic cleavage, ii) a proportion of these these fragments may be taken up locally in fibroblasts or other cell types and processed intralysosomally, iii) a considerable proportion of the fragments follow the lymph flow to local lymph nodes, where specialized lymph node scavenger cells endocytose and degrade some, iv) finally, those CTM degradation fragments that make it passed the lymph nodes will appear in the circulation, from where they are removed by high capacity and specificity endocytosis in the liver sinusoidal endothelial cell (LSEC). Of note, collagen fragments released from bone (where most of the body collagen resides), will not be drained by lymph, but appear directly in the blood circulation. Therefore, LSECs appear to play a particularly important role in removal of collagen fragments from the circulation. The role of the LSEC in the systemic metabolism of CTMs is not yet fully accepted. In discussions on this topic it is still common to use the following argument against the importance of clearance of these structures from the circulation: "The fact that the serum concentration of most CTMs is so low - sometimes below the detection limit of most assay types, will point against the need for a very active uptake system for these molecules in the LSECs." The simple response to this kind of argument will be: "Turn the argument around - the very low serum concentrations of these molecules reflects the very important uptake activity in LSECs."

What are the special features of LSECs that endow them with their remarkable capacity and specificity required for efficient clearance of blood borne CTMs? To answer this question the two following facts should be appreciated: i) The liver receives roughly 25-30% of the blood pumped by the heart, and all this blood passes through the hepatic sinusoids, where the LSECs make up the lining, enabling them to screen the blood contents in a most efficient manner. ii) The LSECs represent a recently discovered group of vertebrate scavenger endothelial cells (SECs) that play an important role in the innate immune system, eliminating an array of own and foreign waste macromolecules and colloids. In fact, these cells stain positively with the vital stains that were used during the decades before and after 1900 to identify the so called Reticuloendothelial System (RES). Although not yet included in text book literature, research over the past 2-3 decades has shown that the backbone of RES is LSECs, with their scavenger function being restricted to non-phagocytic clathrin-mediated endocytosis of macromolecules and colloids (< 200 nm), and Kupffer cells playing an important scavenger role being specialized on phagocytic uptake (particle size >200 nm). We have shown that all major CTMs and their macromolecular fragments are cleared from the
circulation by receptor-mediated endocytosis mainly in LSECs. Moreover, work in our laboratory and our collaborators’ laboratories have shown over the last decade that the LSEC express 3 unique endocytosis receptors that enable their unsurpassed uptake of blood borne soluble macromolecular waste: i) Stabilin-2 = class H scavenger receptor = the major scavenger receptor of LSEC; ii) the mannose receptor (the macrophage mannose receptor C type i, MRC1, or CD 206), and iii) the FcgIIb receptor (FcgIIbR = CD32b = SE-1). Of note, these are all receptors that – according to the existing but erroneous understanding of RES – are mistakenly believed to be expressed mainly in macrophages, and thus in Kupffer cells. However, our findings have changed this paradigm, and the correct notion should be that these receptors are expressed in LSECs, but not – or only to a minimal extent - in Kupffer cells. Our findings have revealed that only two of these unique endocytic LSEC receptors are in operation to clear blood borne CTMs:

1. **MRC1.** This receptor has a dual binding specificity: i) a C-lectin type domain binds mannose (and ManNAc and GlcNAc) carried in the terminal glycan structures of the ligand, and ii) a non-lectin type collagen alpha chain binding domain. With these two distinct ligand binding domains the LSEC MRC1 mediates the clearance of C-terminal propeptides of type I procollagen, whereas alpha chains of several types of collagen are cleared by the collagen alpha chain binding domain.

2. **Stabilin 2 (and to some extent stablolin 1).** This receptor represents the unique LSEC scavenger receptor, and mediates the clearance of negatively charged macromolecules, such as hyaluronan, chondroitin sulphate, nidogen, and N-terminal propeptides of types I and III procollagen.

Following unusually rapid internalization via the LSEC Stabilin 2 and MRC1, the CTMs are transferred to endo/lysosomal organelles and rapidly degraded to low molecular weight end products. Interestingly, LSECs operate on anaerobic metabolism, and their endocytosis of massive amounts of blood borne macromolecular waste results in the production of large amounts of lactate. We have hypothesized that this lactate is utilized as readily accessible fuel for the production of ATP in the highly metabolic active hepatocytes.

In conclusion: The role of LSECs in the catabolism of connective tissue is to rid the blood of CTM fragments originating in peripheral tissues. LSECs degrade the internalized CTMs to low molecular products that are either excreted by kidneys glomerular filtration, or reutilized by the metabolically active hepatocytes that are in constant need of energy.