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Overview of Extracellular Microvesicles in Drug Metabolism

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Abstract

Importance of the field—Liver is the major body reservoir for enzymes involved in the metabolism of endogenous and xenobiotic compounds. Recently, it has been shown that hepatocytes release exosome-like vesicles to the extracellular medium, and the proteomic characterization of these hepatocyte-secreted exosomes has revealed the presence of several of these enzymes on them.

Areas covered in this review—A systematic bibliographic search focus on two related aspects: 1) xenobiotic-metabolizing enzymes that have been detected in microvesicles, and 2) microvesicles which are in the blood stream or secreted by cell-types with clear interactions with this fluid.

What the reader will gain—A discussion of these hepatocyte-secreted vesicles along with others microvesicles as enzymatic carriers in the context of extrahepatic drug-metabolizing systems.

Take home message—The contribution of many tissues including the liver to the microvesicles plasma population is supported by several reports. On the other hand, many enzymes involved in the metabolism of drugs have been detected in microvesicles. Together, these observations argue positively through a role of hepatic-microvesicles in spreading the liver metabolizing activities through the body contributing in this manner to extrahepatic drug metabolism systems what could be relevant for body homeostasis and pharmaceutical interests.

Keywords

Cytochromes; Drug-metabolism; Extrahepatic; Exosomes; Microvesicles

1. Introduction

The major research efforts in the field of “xenobiotic-metabolizing enzymes” have been concerned with systems within the liver. This organ helps to protect the organism against hundreds of exogenous chemical substances found in the environment and in our daily diet, including pharmaceutical drugs used for the treatment and prevention of diseases [1]. Drugs metabolized in the liver are distributed by the blood to the different tissues and organs of the organism. However, metabolism in other tissues apart from the liver also occurs and drug metabolizing enzymes have been identified in almost every tissue. The expression of these

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enzymes in extrahepatic tissues may not be as high as the liver, but they have been shown to play pivotal roles in the metabolism of specific endogenous compounds as well as drugs [2]. Recently, it has been shown that hepatocytes release exosome-like vesicles to the extracellular medium, and the proteomic characterization of these hepatocyte-secreted exosomes has revealed the presence of several of these enzymes on them. In this review an overview of drug-metabolizing enzymes detected in microvesicles (Table 1) precedes to the main topic which discusses the possible implications of extracellular microvesicles in drug metabolism and integrates the few reported data that are available so far regarding extracellular microvesicles associated with drug metabolism and development of drug resistance.

2. Drug Metabolizing Enzymes

Most drugs are metabolized by the so-called “xenobiotic-metabolizing”, “drug-metabolizing” or “toxification-detoxification” systems. These systems are composed mainly by two different types of enzymes: enzymes that induce chemical changes in the compound to make it less lipophilic and more hydrophilic (phase I metabolism); and enzymes that are capable of forming adducts with the metabolite to facilitate its clearance from circulation (phase II metabolism). Phase I reactions include oxidation, reduction, and hydrolysis processes which introduce or modify functional groups (such as hydroxyl groups) in the compound to make it more soluble or suitable for phase II reactions. Phase II metabolism consists of conjugation reactions involving a large variety of moieties to facilitate the excretion of the compound. A phase III reaction is often referred to the transport of reactive metabolites across cellular barriers to help its excretion and elimination from the organism. After being processed by these systems, the by-product metabolites are mostly excreted into the urine, while minor fractions which include volatile compounds are often excreted via the lungs, skin, saliva, etc [3].

Cytochromes P450 (P450s) constitute the largest family of xenobiotic compound metabolizing enzymes localized in several hepatic and non-hepatic tissues. In humans, 57 enzymes have been identified and collectively have more substrates than any other enzymes [4]. P450s are monooxygenases that catalyze phase I reactions, and consist of an apoprotein and a heme moiety which is common to all cytochromes [5–7]. P450s are involved in a broad range of reactions including hydroxylation, N-, O-, and S-dealkylation, sulfoxidation, epoxidation, deamination, desulphuration, dehalogenation, peroxidation, and N-oxide reduction [8]. The levels of these few enzymes vary widely among individuals, found sometimes to be completely absent. Due to the extent of this variability, the desired pharmacological effect is sometimes not observed upon drug administration with the consequent side effects [4]. Generally, all mammalian P450s are anchored to the membrane through a hydrophobic protein surface and a transmembrane domain at the end of the N terminus. The rest of the protein, including the catalytic domain, is exposed to enable the reaction with substrates [9]. Even though P450s primarily reside in the endoplasmic reticulum, they have also been observed in many other organelles including the inner mitochondria membrane, the plasma membrane, the outer nuclear membrane, Golgi apparatus, lysosomes, and peroxisomes. Each P450 commonly resides in a single sub-cellular compartment, but the presence of the same cytochrome in several organelles have also been observed [10]. The organelle in which the P450s reside may reflect important characteristics that distinguish them from others. An example is found in mitochondrial P450s which differ from their microsomal counterparts in their substrate specificity. While microsomal P450s metabolize principally xenobiotic compounds, the mitochondrial enzymes have been shown to metabolize mainly endogenous compounds such as vitamins and steroid hormones [9].

Another major contributor to drug metabolism is the family of uridine dinucleotide phosphate (UDP) glucuronosyl transferases (UGTs). Approximately 20 different UGTs have been identified in human. Many of these are expressed in the liver, but they have also been identified

in other tissues such as intestine, brain, kidney, and skin. UGT enzymes are membrane bound proteins found primarily in the endoplasmic reticulum of cells. A wide range of drugs as well as other xenobiotics, including environmental and dietary xenobiotics, have been shown to be metabolized by these enzymes. [11]. UGTs catalyze phase II glucuronidation reactions in which a glucuronic acid moiety is transferred from the substrate UDP-glucuronic acid to lipid-soluble molecules to form more hydrophilic substrates which can be easily excreted in the urine and bile [12] [13]. UGTs are usually located in close proximity to P450s in the endoplasmic reticulum in which the catalytic domains of both types of enzymes are facing the cytosolic and luminal sides, respectively. These two types of enzymes present broad, overlapping substrate specificities. These proteins have been shown in several studies to interact with each other and be functionally active [14–18]. It is generally thought that a coordinated biotransformation reaction occurs between both drug metabolizing enzymes. These protein interactions would permit a more efficient metabolism of a compound modulating enzyme activity and substrate specificity [19].

Glutathione S-transferases (GSTs) are another important family of enzymes catalyzing phase II reactions to be considered in the metabolism of drugs. Soluble and membrane bound forms exist in this family of proteins. Eight different gene families encode for GSTs in humans. The general reaction of GST enzymes is the addition of glutathione (GSH), a nucleophilic tripeptide present in the cellular cytosol, to a wide variety of endogenous and exogenous substrates [20]. GSTs are commonly responsible for the metabolism of oxidation products such as epoxides derived from the metabolism of P450s on polycyclic aromatic hydrocarbons. The addition of GSH moieties to these metabolically reactive epoxides turns them up into inactive forms that are readily excretable from the body [21]. However, the mediated conjugation of GSH to xenobiotic compounds by GSTs has also been observed to yield metabolites that are cytotoxic and if not detoxified, may lead to various forms of hepatic and extrahepatic toxicity, including cellular necrosis, hypersensitivity, teratogenicity, and carcinogenicity, depending on the site of formation and the relative stability of the metabolite, and the cellular macromolecule with which it reacts [22].

Many other types of phase I enzymes are involved in the metabolism of drugs and other xenobiotics but these have fewer substrates and carry out a markedly narrower range of reactions. For instance, alcohol and aldehyde dehydrogenases catalyze the oxidation of ethanol to acetaldehyde and then to acetate [23]. Aldo-keto reductases are involved in the oxidation-reduction of aldehydes and ketones either as endogenous molecules such as glucose and glucocorticoids or exogenous compounds including drugs. These proteins can either function independently or in collaboration with other enzymes including alcohol and aldehyde dehydrogenases, P450s, and GSTs [24]. Flavin-containing monooxygenases have been long overlooked because some of the metabolic reactions carried out by these enzymes often yield the same metabolites than P450s. However, a major difference between P450s and the latter is that these have not been shown to be either induced or inhibited by drugs or other xenobiotic compounds, resulting in a low potential for drug-drug interactions [25]. Hydrolysis reactions are also to be considered in the metabolism of drugs. In this sense, there is an immense variety of hydrolases but the most significant ones regarding xenobiotic metabolism are carboxylesterases, cholinesterases, arylesterases, paraoxonases, and epoxide hydrolases [26]. These esterases play an important role in the detoxification processes that take place in serum [27]. An example is the metabolism of aspirin, only 30% of an oral dose is hydrolyzed to salicylate in its pass through the gut and the liver, and the rest is rapidly hydrolyzed by serum esterases once it reaches the circulation [28]. Cholinesterases and aryl esterases seem to be the main hydrolases in the detoxification function of serum. There are two types of cholinesterases: the first is highly specific for acetylcholine and it is often referred as acetylcholinesterase; the second is capable of hydrolyzing both choline and aliphatic esters and is termed pseudocholinesterase.

Pseudocholinesterase is synthesized in the liver and secreted into the plasma while acetylcholinesterase is commonly part of the plasma membrane of erythrocytes, and tissues such as nerve and muscle [29]. There are several studies supporting the action of plasma cholinesterase against a large number of drugs. For instance, suxamethonium, a neuromuscular blocking agent, was found to have a very short action because it was rapidly hydrolysed by plasma cholinesterase [30]. Other anesthetic drugs that have been found to be metabolized by plasma cholinesterase include mivacurium [31], cocaine [32], and diamorphine [33], as well as other drugs such as the glucocorticoid methylprednisolone acetate [34] which is activated upon its hydrolysis. Apart from a multiplicity of functions, albumin is known as one of the major carriers of drugs throughout the body as well as other endobiotic compounds. It is the most abundant protein in plasma and it is synthesized in the liver. Albumin has been shown to bind a large number of drugs and this property have been largely exploited to use albumin as a drug delivery system [35]. Plasma albumin may also act as an esterase under certain circumstances. It has been reported to hydrolyzed many different compounds such as esters, amides, and phosphates [36,37] suggesting that plasma albumin plays an important role in the metabolism of drugs [38,39].

3. Hepatic and Extrahepatic Microvesicles in Drug Detoxification

Small membranous vesicles of different origins that are referred to as microvesicles (MVs) [40] have been reported to be part of the plasma content (Figure 1). For a long time MVs were believed to be artefacts resulting from the cellular lyses, but since their biological relevance have been demonstrated in many different processes such as coagulation, immunological responses and tumour progression, these vesicles are “artefact no more” [40,41]. Depending mainly on the vesicle’s origin and the way of vesicle-discharge from the cells, two types of microvesicles have been described, the endosome-derived vesicles named “exosomes” and the plasma membrane shedding vesicles referred as microparticles. Exosomes are the type of vesicles better defined so far. With a size of 30–150 nm, they have an endocytic origin, are formed by inward budding of the membrane of an endocytic organelle named multivesicular body and released to the extracellular space by fusion of this organelle with the plasma membrane [42]. Microparticles, on the other hand, are considered to be a more heterogeneous and less known group so that a consensus is not still reached in nomenclature of its integrating components. With a size up 1000 nm, this group consists of vesicles that are formed directly from plasma membrane by so-called reverse budding involving membrane protrusion and fission [43–45]. The content of microvesicles and their biological function depend on the cell-type origin and common as well as cell-type specific proteins have been detected in them. Interestingly, MVs besides containing lipids and proteins, also give refuge to mRNA, small RNAs including miRNA [46–50], mtDNA [51] and even genomic DNA [52]. Transference of this material takes place and is functional in the recipient cell [47,49,50] indicating that these vesicles are involved in transport of material from one cell to another. Due to this feature, MVs are being studied by many groups as possible vehicle to direct specific cargos to target cells [50,53], or as carriers of antigens for vaccination [54–56]. Although, the cell biology of these two types of microvesicles -exosomes and microparticles- is quite different, upon release from the cells, both types circulate in the extracellular space adjacent to the cell of origin and by still an undefined mechanism can move and appear in the blood. Microvesicles have been found in human, rodent, and foetal calf sera [57–62], and demonstrated to be released by both haematological and non-haematological-originated [40,41], quiescent or activated [63], non-transformed or tumoral cells [64]. Nowadays, it is thought that between 70–90% of these vesicles in plasma are blood cells-originated, in particular from platelets and endothelial cells [61,63]. However, many studies show the presence of serum microvesicles of different origin [44,50,57,62,65–87] (Figure 1). For example, Masciopinto *et al.* reported that exosomes from serum of Hepatitis C patients carried RNA molecules from the virus [79], suggesting that they could be originated in the liver. Witek *et al.* have recently showed that cholangiocytes and

myofibroblastic hepatic stellate cells, two of the cell populations that are part of the liver, release microparticles containing active Hedgehog ligands in response to platelet derived growth factor, and their levels increase in the serum in response to liver injury [87]. Other MVs secreted by cells from hepatic origin have also been reported including hepatoma cell lines such as HepG2 [79,88], Huh7 [79], non-tumoral liver-derived cell lines such as MLP29 and primary cultured hepatocytes [69].

A few reports have highlighted the relevance of these MVs in drug metabolism. Johnstone *et al.* in the late 80's performed characterization of a major route for shedding of plasma membrane functions during the maturation from reticulocytes to erythrocytes [75,89]. During this maturation process reticulocytes remove a large number of organelles including mitochondria and nucleus, and substantial changes in the protein composition of the plasma membrane also occur. Johnstone *et al.* showed that many of the dispensable proteins are eliminated by releasing in MVs. They characterized these vesicles and found several enzymatic activities that were associated with them, interestingly; by measuring the hydrolysis of acetylcholine they were able to detect acetylcholinesterase activity. The fact that this activity has been shown to metabolize a few drugs such as butyrylcholine constitutes one of the first association of these plasma microvesicles with drug metabolism. Another set of evidences supporting a role of these microvesicles in drug metabolism are provided by several proteomic works which have revealed the presence of different xenobiotic metabolizing enzymes on them. Thus, proteomic analysis of microvesicles directly purified from the plasma as well as derived from distinct blood cell types have detected a variety of drug metabolizing enzymes on them (Table 1). Jin and collaborators identified 169 spots –corresponding to 83 different proteins– out of 1055 different spots from a two-dimensional SDS-PAGE analysis of human plasma microparticles, representing only a 16 % of the detected-protein spots by sypro gel staining [74]. In this 16% at least 7 proteins were associated with drug metabolism including glutathione transferase and albumin (Table 1). Recently, a study of human plasma microvesicles performed by one-dimensional SDS-PAGE combined with mass spectrometry has identified 66 proteins some of them related with drug metabolism [77] (Table 1). Taken into account that so far a small proportion of the protein content of plasma microvesicles has been identified it can not be exclude the presence of additional drug metabolism-related proteins in these vesicles. Indeed a comprehensive proteomic study combining one dimensional SDS-PAGE and mass spectrometry analyses of highly-purified microvesicles secreted by primary hepatocytes has revealed the presence of several members of the P450s, UGTs and GSTs drug-metabolizing protein families (Table 1) [69]. Given the existence of these hepatocyte-derived microvesicles carrying P450s, UGTs and GSTs and the narrow interface between liver and blood, it is reasonable to assume that these enzymes will be mobilized to the blood and circulate in the body. The detection by western-blot analysis of cytochrome CYP17A1 in microvesicles purified from human serum supports the occurrence of this mobilization process [90], and also shows that new proteomic approaches need to be undertaken to extent our knowledge of protein content of plasma microvesicles.

Finally, it is worth mentioning the role that microvesicles play in the development of drug resistance to antitumoral compounds by a metabolism independent mechanism involving the sequestration of the drug into intracellular vesicles that are secreted from the cell as microvesicles. Safaei *et al.* have shown that the drug cisplatin (CDDP) is secreted via exosomes along with putative CDDP transporters in a human ovarian carcinoma cell line named CDDP-resistant 2008/C13*5.25 [91]. CDDP is a platinum-based chemotherapy drug used to treat various types of cancer which induces resistance in tumor cells upon repeated exposure. According to the results reported by Safaei *et al.*, exosomes release could be one of the mechanisms responsible of drug resistance. In other study, accumulation of doxorubicin and other anti-cancer agents in the membrane of MVs have been shown to be accompanied by a decrease of their corresponding cellular levels and contribute to drug resistance [92].

Furthermore, P-glycoprotein (P-gp) was found to be transferred between drug-resistant cancer cells and drug sensitive cells via microparticles [93]. P-gp is an ATP-binding cassette (ABC) transporter involved in multidrug resistance (MDR) that is able to recognize and transport a plethora of substrates. MDR is a condition enabling a cell to resist drug treatment. P-gp has been found to be over-expressed in cancer cells where it helps to maintain sub-lethal intracellular drug concentrations by an increased drug efflux, often leading to the development in these cells of resistance to anticancer drugs [94]. In the study, P-gp was shown to be present on microvesicles. Using the drug-resistant cancer VLB (100) cell line, functional P-gp was shown to be transferred to CCRF-CEM cells, a drug sensitive cell line, after co-culture. These studies suggest a possible role of microvesicles in the acquisition and spread of MDR, which has been shown to be an impediment to the successful treatment of cancer clinically.

4. Expert opinion

The fact that it has been shown that hepatocytes are able to secrete microvesicles to the extracellular medium containing a high number of P450s, UGTs and other liver proteins involved in xenobiotic metabolism (Table 1) [69] indicates that these hepatic microvesicles may play a role in the metabolism of endogenous and xenobiotic compounds and help to control the homeostasis of the body. However some issues need to be addressed in order to confirm this possible contribution of these vesicles to the hepatic and extrahepatic metabolizing systems.

- i. Are these enzymes active in these vesicles? A precedent with exosomes derived from erythrocytes argues favorably to a positive answer to this question. As already mentioned Jonhstone *et al.*, were able to detect the activity of acetylcholinesterase in those exosomes [75,89]. Further studies characterizing the activity of other enzymes such as P450s, UGTs and GSTs which are the enzymes mainly found in hepatocyte-secreted exosomes are necessary.
- ii. Are these hepatocyte-derived exosomes able to spread beyond the liver? Pending of additional experiments, two arguments are in favor:
 - a. In the liver architecture the hepatocyte milieu is separated from blood by a line of fenestrated endothelial cells with a diameter pore that vary depending on the physiological conditions between 50 and 200 nm in size [95]. This diameter allows the transit of vesicles of less than 200 nm in size and hepatocytes-derived vesicles have a mean size of 60 nm as determined by cryo-electron microscopy [69].
 - b. The presence of these hepatocyte-secreted vesicles in the plasma is supported by results obtained by Brodsky *et al.* They have isolated plasma circulating microvesicles enriched in those liver-originated ones by using an antibody specific to the relatively liver-specific enzyme carbamoyl phosphate synthetase 1 [68,96]. This enzyme is also highly represented in the proteomic characterization of hepatocyte-derived exosomes [69], indicating that part of the microvesicles immunoisolated by Brodsky *et al.* may correspond to the hepatocyte-derived extracellular vesicles.
- iii. What are the physiological implications of these hepatic-secreted vesicles? Several speculative roles can be envisioned at present.
 - a. Besides their participation in the metabolism of endogenous and xenobiotic compounds, these vesicles could be part of the mechanism mobilized to response against a poisoning condition. The poison could induce in the liver the expression of the specific poison-metabolizing enzyme, which after be incorporated into microvesicles is spreading through the body to help and

accelerate its clearance. To establish this intriguing role it will be necessary to study both the amount and the composition of these vesicles in blood samples from animal models before and after poison exposure.

- b. These microvesicles could have also implications in the development of therapeutic drugs. The possible contributions of these secreted vesicles to the metabolism of drug will need to be taken in account to avoid undesired secondary effects. In this sense it will be interesting to analyze the implications that these vesicles could have in idiosyncratic drug toxicity, which includes unexpected and relatively rare host responses to therapeutic treatment or chemical exposure by mostly unknown mechanisms [97].
- c. Another possibility is that these microvesicles could be entirely unrelated to a metabolic purpose and the presence of the drug metabolizing enzymes in them is co-incidental due to the process of microvesicles formation. Alternatively, these vesicles can also constitute a mean to remove from the liver the inactivated or damaged enzymes, in order to avoid liver injury. In this context is worthy to mention the possible implication of these microvesicles in the development of autoimmune hepatitis, a liver disorder of unknown etiology and pathogenesis. In patients with autoimmune hepatitis, disease-specific autoantibodies are frequently directed against drug metabolizing enzymes such as P450s and UGTs [98,99]. How these enzymes become “self targets” is not yet established. One possible explanation could be that as a result of the reaction with the drug, the enzymes can be inactivated or damaged by covalent-modifications generating in consequence new epitopes. The secretion of these covalent-modified proteins via these microvesicles could expose the neoepitopes to the immune system inducing an immune response towards these enzymes causing liver injury.
- d. A final but important implication of these microvesicles in individualized therapy is given by the different efficiency that xenobiotic metabolizing enzymes display among individuals and populations [1]. In this sense hepatocyte-derived exosomes purified from blood sample of a patient could be utilized to extrapolate in a non-invasive way how his or her liver or body is going to metabolize a specific drug helping to the physician to establish the best treatment possible.

Although the knowledge about the physiological role of these hepatocytes-derived vesicles is in its infancy, there is no doubt that just the realization of their existence has opened many interesting issues that may have a future impact in clinical and pharmaceutical applications.

Box 1

Microvesicles are extracellular vesicles secreted by numerous cell-types to the extracellular media. They have been shown to transfer proteins and RNA between different cells, to function in coagulation, immunological responses and tumoral progresion, and depending on their origin to content different proteins and enzymes implicated in drug detoxification.

Abbreviations

P450s	Cytochromes P450
UGTs	UDP-glucuronosyl transferases

GSTs	Glutathione S-transferases
MVs	Microvesicles
MDR	Multidrug resistance

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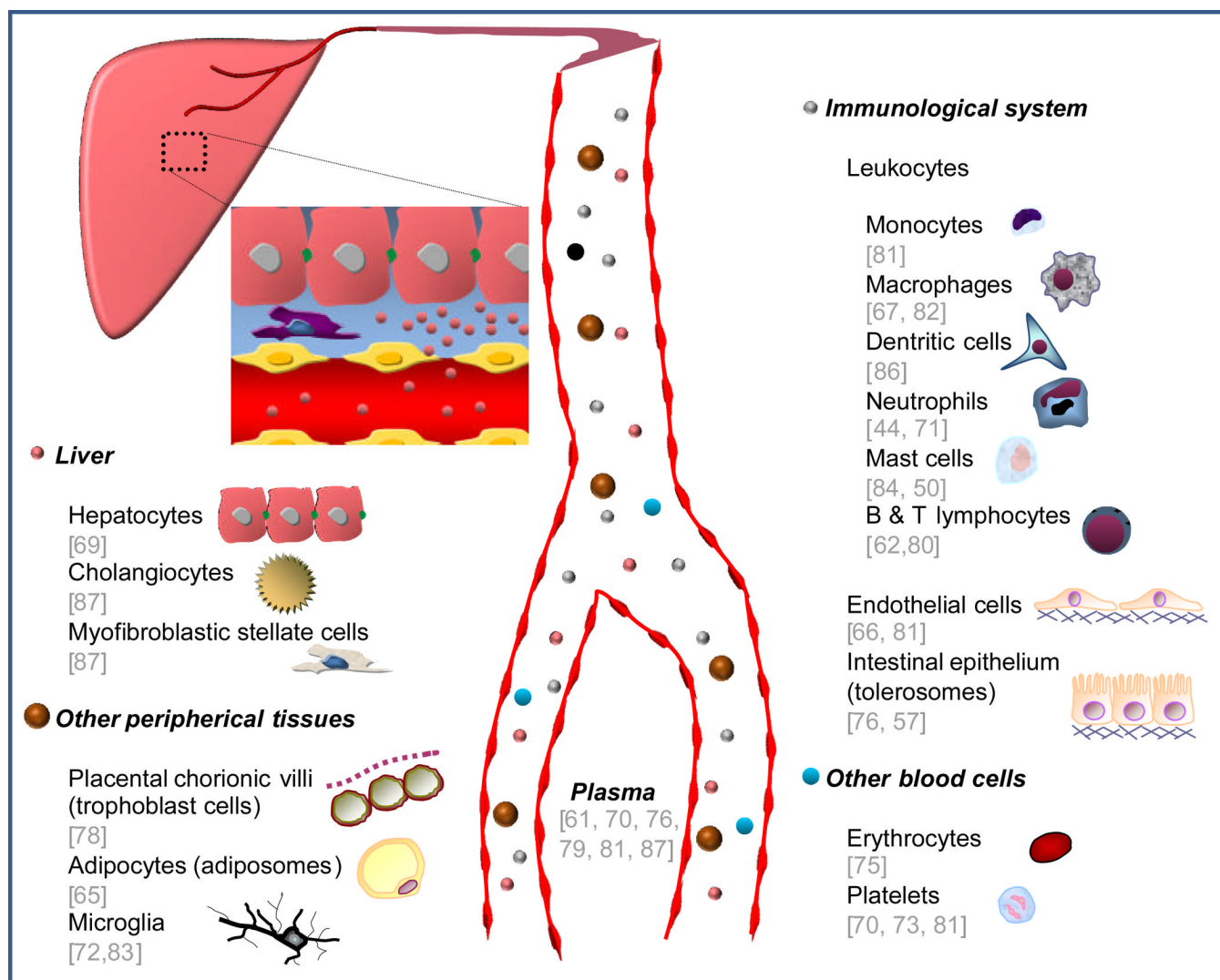


Figure 1.

Schematic view of the complex mixture of microvesicles originated from different tissues and cell-types that are circulating in the blood stream. Different cell-types involved in immune response have been shown to secrete microvesicles (*gray balls*). Others blood cells including erythrocytes and platelets are known to contribute to the microvesicles plasma population (*blue balls*). Hepatocytes are one of the hepatic cell-types along with cholangiocytes and myofibroblastic stellate cells that secrete microvesicles that possibly are mobilized to the blood stream (*pink balls*). Other growing group of peripheral tissues including neuronal and adipose tissue have also been reported to release these microvesicles that are mobilized to the blood stream (*brown balls*). Several works have described microvesicles directly isolated from plasma. References are indicated in brackets.

Table 1

Proteins associated to the metabolism of endogenous and xenobiotic compounds detected in microvesicles.

Origin	Ref.	Purification and identification Procedure [#]	Enzyme Description
Rat Hepatocyte	[69]	0.22µm filtration	Serum Albumin
		Differential	
		ultracentrifugation	Serum paraoxonase/lactonase 3
		Sucrose cushion	Serum paraoxonase/aryesterase 1
		SDS-PAGE	Cytochrome P450 2A1
		LC-MS/MS	Cytochrome P450 2A2
			Cytochrome P450 4A2
			Cytochrome P450 2B3
			Cytochrome P450 2C11
			Cytochrome P450 2D1
			Cytochrome P450 2D3
			Cytochrome P450 2D18
			Cytochrome P450 2D10
			Cytochrome P450 2D26
			NADPH--cytochrome P450 reductase
			UDP-glucuronosyltransferase 1-6
			UDP-glucuronosyltransferase 1-8
			UDP-glucuronosyltransferase 2B1
			UDP-glucuronosyltransferase 2B2
			UDP-glucuronosyltransferase 2B3
			UDP-glucuronosyltransferase 2B5
			Aldehyde dehydrogenase X
			Aldehyde dehydrogenase, mitochondrial
			Aldehyde oxidase
			Alcohol dehydrogenase 1
			Carboxylesterase 3
			Epoxide hydrolase 1
			Aflatoxin B1 aldehyde reductase memb 2
			Aflatoxin B1 aldehyde reductase memb 3
			Microsomal glutathione S-transferase 1
			Glutathione S-transferase Mu 1
			Sulfotransferase 1A1
			Thiosulfate sulfurtransferase
			Dimethylamine monooxygenase
			Dimethylaniline monooxygenase
			Carbonic anhydrase 3
			Catalase
			Peroxiredoxin-1

Origin	Ref.	Purification and identification Procedure [#]	Enzyme Description
Sheep Reticulocytes	[75]	Differential	Catechol O-methyltransferase
		ultracentrifugation	4-aminobutyrate aminotransferase, mito.
		Activity assay	Acetylcholinesterase
Human Platelets	[70]	ADP treatment	Serum Albumin
		Differential	
		ultracentrifugation	Glutathione transferase
		SDS-PAGE	Glutathione reductase
		LC-MS/MS	Glutathione synthetase
			Glutathione peroxidase 1
			Carbonic anhydrase I
			Carbonic anhydrase II
			Cytochrome b5 reductase membrane-bound isoform
			Catalase
			UDP-glucose ceramide glucosyltransferase-like 1
			Prolyl 4-hydroxylase, beta subunit
			Thiosulfate sulfurtransferase; rhodanese
			Aldo-keto reductase 7A2
			Peroxiredoxin 1
			Peroxiredoxin 2
			Peroxiredoxin 3
			Thioredoxin peroxidase
			Peroxiredoxin 5
			Peroxiredoxin 6
			Esterase D/formylglutathione hydrolase
			S-adenosylhomocysteine hydrolase
			Monoamine oxidase B
			Esterase D
			Carbonyl reductase 1
			Alcohol dehydrogenase class III
			Cytochrome b5 reductase membrane-bound isoform
			Thiosulfate sulfurtransferase; rhodanese
			Manganese superoxide dismutase
Human Plasma	[77]	0.22µm filtration	Serum Albumin
		FPLC	Peroxiredoxin 2 isoform b
		Continuous sucrose gradient	Paraoxonase 1
		SDS-PAGE	
		LC-MS/MS	

Origin	Ref.	Purification and identification Procedure [#]	Enzyme Description
Human Plasma	[74]	Differential	
		ultracentrifugation	Serum Albumin
		2-D gel	
		electrophoresis	Glutathione peroxidase 1
		MALDI-MS/MS	Glutathione S-transferase P
			Peroxiredoxin 1
			Peroxiredoxin 2
Human Serum	[90]		Peroxiredoxin 3
			Superoxide dismutase [Mn]
		Differential	
		ultracentrifugation	Cytochrome CYP17A1
		Western-blotting	

[#] Abbreviations: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; LCMS/MS, Liquid Chromatography-Tandem Mass Spectrometry; ADP, Adenosine diphosphate; FPLC, Fast Protein Liquid Chromatography; MALDI-MS/MS, Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry