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GUEST EDITORIAL

Connexin hemichannels: novel mediators of toxicity

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Tissue homeostasis is driven by a myriad of extracellular, intracellular and intercellular signaling networks. Direct communication between neighboring cells is typically controlled by gap junctions (Decrock et al. 2009; Nielsen et al. 2012; Vinken et al. 2011). Gap junctions mediate the intercellular diffusion of small and hydrophilic substances, including cyclic adenosine monophosphate, adenosine triphosphate, inositol triphosphate, glutathione, glutamate, glucose and several ions (Alexander and Goldberg 2003) (Fig. 1a). This flux is denoted as gap junctional intercellular communication (GJIC) and is considered as a key mechanism in the maintenance of tissue functioning (Decrock et al. 2009; Nielsen et al. 2012; Vinken et al. 2011). Over the last decades, GJIC has been shown indispensable for the establishment of metabolic or electrical intercellular coupling in all vital organs, such as the brain (Eugenin et al. 2012), the heart (Kurtenbach et al. 2014) and the liver (Vinken et al. 2008). Gap junctions are formed by the docking of two hemichannels of adjacent cells, which in turn are composed of six connexin proteins. At present, 21 different connexins have been identified in humans, all which are expressed in a cell-specific way (Kar et al. 2012; Nielsen et al. 2012). They are named based upon their molecular weight. Thus, the most widespread connexin species has a molecular mass of 43 kDa and hence is called Cx43. Connexin proteins share a common molecular structure consisting of four transmembrane domains, two extracellular loops, one cytosolic loop, one cytosolic aminotail and one cytosolic carboxytail (Fig. 1b) (Decrock et al. 2009; Nielsen et al. 2012).

Although considered as merely structural precursors of gap junctions for a long time, an abundance of reports published in the last few years shows that connexin hemichannels as such can provide a pathway for cellular communication, albeit between the cytosol of individual cells and their extracellular environment and not between adjacent cells as is the case for GJIC. Nonetheless, the messengers that are conveyed through connexin hemichannels are very similar to those involved in GJIC, including adenosine triphosphate, nicotinamide dinucleotide, glutamate, glutathione, prostaglandin, sodium and calcium ions (Fig. 1a) (Chandrasekhar and Bera 2012; Decrock et al. 2009; Kar et al. 2012). Furthermore, connexin hemichannels are regulated by mechanisms that equally affect gap junctions, yet an identical factor can have opposing effects on the two channel types, such as shown for certain inflammatory triggers (De Vuyst et al. 2007; Retamal et al. 2007). In line with this notion, connexin hemichannels, unlike their full channel counterparts, display a low open probability. In fact, connexin hemichannels seem to be preferably activated by pathological stimuli, including ischemia–reperfusion insults and oxidative stress, and thereby drive processes like cell death and inflammation (Chandrasekhar and Bera 2012; Decrock et al. 2009; Kar et al. 2012). For this very reason, connexin hemichannels are sometimes considered as "pathological pores" (Decrock et al. 2009). This concept is clearly relevant to toxicologists, as several toxic substances open up connexin hemichannels. For example, ultrafine carbon black particles activate Cx43-based hemichannels in astrocytes, which may represent a novel mechanism of neurotoxicity (Wei et al. 2014). Similarly, cobalt–chromium nanoparticles can damage human fibroblasts by a mechanism involving transmission of adenosine

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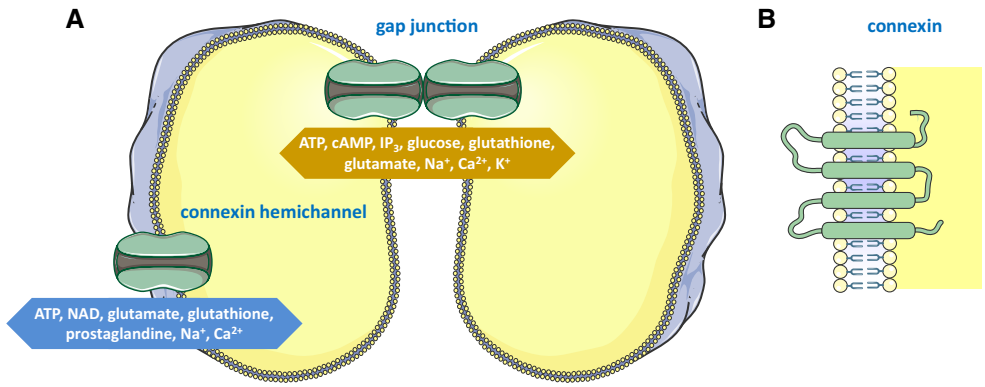

Fig. 1 **a** Gap junctions consist of two connexin hemichannels of adjacent cells and mediate intercellular communication. Connexin hemichannels also provide a pathway for communication, namely between the cytosol and the extracellular environment. Similar messengers travel through gap junctions and connexin hemichannels. **b** Connexin proteins have four transmembrane domains, two extracellular loops, one cytosolic loop, one cytosolic aminotail and one cytosolic carboxytail (*ATP* adenosine triphosphate, *cAMP* cyclic adenosine monophosphate, IP_3 inositol triphosphate, *NAD* nicotinamide dinucleotide)

triphosphate and signaling through connexin hemichannels (Bhabra et al. 2009).

The connexin research field has been surrounded by a lot of controversy in the last few years. Specifically, the concept of (dys) functional connexin hemichannels has been debated heavily on several occasions (Sáez and Leybaert 2014; Spray et al. 2006). A major reason for this impediment is the ubiquitous lack of tools and technologies to distinguish between the different channel types, in casu between gap junctions and connexin hemichannels (Bodendiek and Raman 2010; Iyyathurai et al. 2013). Classical strategies, such as the use of RNA interference-based technologies, genetically modified animals or even antibodies, are indeed not applicable, as they target connexins, which are the shared building stones of gap junctions and connexin hemichannels. Furthermore, most, if not all, of the routinely used gap junction inhibitors, including long-chain alcohol substances, anaesthetic substances, the glycyrrhetinic acid derivative carbenoxolone and the fenamate family of blockers, equally suppress connexin hemichannel activity (Bodendiek and Raman 2010). Great expectations now lie with peptides that reproduce sequences in the cytosolic loop regions of connexins, as they suppress connexin hemichannel activity without influencing GJIC (Iyyathurai et al. 2013). Among those, a very prominent one is Gap19, a peptide that inhibits Cx43-based hemichannels while leaving the corresponding gap junctions unaffected (Abudara et al. 2014; Wang et al. 2013). In fact, Gap19 was found to reduce experimentally induced cell death in a mouse model of cardiac ischemia–reperfusion (Wang et al. 2013). This area is still in its infancy, but upon further exploration, it can be expected that this will also open new perspectives for the field of toxicology. Indeed, such specific connexin hemichannel inhibitors are not only interesting experimental tools to shed light onto yet unraveled toxicological mechanisms of action, but they may also form the basis for the development of antidotes pertinent for clinical use.

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