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COMMENTARY

ZP1609/danegaptide and mitochondrial connexin hemichannels: a harbinger for peptide drug design

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LINKED ARTICLES

This article is a Commentary on Boengler K, Bulic M, Schreckenberg R, Schlüter K-D, Schulz R (2017). The gap junction modifier ZP1609 decreases cardiomyocyte hypercontracture following ischaemia/reperfusion independent from mitochondrial connexin 43. Br J Pharmacol 174: 2060–2073. https://doi.org/10.1111/bph.13804

Over the last 50 years, connexin biologists have greatly expanded the understanding of how cells, tissues and organs coordinate physiological functions through gap junction intercellular connections. One of the most notable examples exists in cardiomyocytes, where connexin 43 (Cx43) gap junctions promote the passing of small ions and molecules between myocytes to coordinate heart muscle contraction. The traditional notion of gap junction formation, especially in cardiomyocytes, begins with the oligomerization of connexin proteins into hexameric connexon units. These assembled units are trafficked to the plasma membrane and inserted into gap junction plaques where they dock with connexons of adjacent plasma membranes. Much of the membrane dynamics and post-translational modifications regulating both gap junction assembly and junctional gating have been extensively studied in a number of physiological contexts (Esseltine and Laird, 2016).

However, there is also a non-canonical view in the connexin field, based on the discovery that some connexons do not enter into docked gap junction plaques but rather exist as functional non-junctional channels called Cx hemichannels (Esseltine and Laird, 2016). Ongoing work focuses on the characterization of single hemichannel properties, and importantly, to distinguish their function from connexons that form gap junctions. Although an accumulation of evidence supports a cellular basis for hemichannel function, much physiological work is left to be explained. For example, concerns over the spatial and temporal localization of Cx hemichannels in native tissues exist, as common optical imaging techniques are challenged to resolve individual hemichannels. Secondly, many functional assays for hemichannel activity utilize cell culture models rather than primary culture or tissue preparations. Thirdly, isolating hemichannel function from gap junction function in cell and animal models has proven difficult as many connexin inhibitors (small molecules and mimetic peptides) lack the targeting specificity to isolate hemichannel function (Esseltine and Laird, 2016). In spite of these shortcomings, Cx hemichannel inhibitors have been used as a basis for altering pathophysiological conditions.

One of the most significant areas of study in which Cx hemichannels have emerged is during ischaemia and reperfusion (I/R) injury in both the brain and the heart (Schulz *et al.*, 2015). In particular, Cx43 formed hemichannels, which are predominantly closed under physiological conditions, apparently open during ischaemia. Inhibition of hemichannel opening has been shown to promote protective effects against I/R injury, notably in cardiomyocytes by ischaemic preconditioning (Schulz *et al.*, 2015). Even more unique is the growing biochemical evidence that supports the localization and possible function of Cx43 hemichannels in the inner mitochondria membrane important during I/R

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injury. Understanding mitochondrial Cx hemichannel function is another area of intense research interest with high therapeutic potential.

Boengler *et al*. (2017) have attempted to understand the importance of targeting both Cx43 gap junctions and/or Cx hemichannels during and after I/R injury in cardiomyocytes. A strong dichotomy appears in I/R injury between blocking Cx43 hemichannels to improve mitochondrial function (i.e. respiration, potassium handling, ROS formation, and mitochondrial permeability transition pore opening), but also stabilizing and promoting Cx43 gap junction conductance to prevent ischaemia-induced arrhythmia (Schulz *et al*., 2015). In this new study, Boengler and collaborators assess the impact of ZP1609, a clinically important dipeptide comprising the Cx43 analogue rotigaptide, specifically on basal mitochondrial function and cardiomyocyte hypercontracture in simulated I/R injury. The basis for these new studies stems from the strong therapeutic effects of ZP1609 in large animal models of I/R (Skyschally *et al*., 2013). In addition, extensive evidence suggests that ZP1609 stabilizes plasma membrane associated Cx43 gap junctions to enhance gap junctional coupling (Kjolbye *et al*., 2007). Given these observations, Boengler *et al*. (2017) attempted to expand *in vivo* and clinical trial observations of ZP1609 protective effects on arrhythmia and reperfusion cell damage. Since Cx43 hemichannels have been detected in only one subset of cardiomyocyte mitochondria, namely, the subsarcolemmal mitochondria (SSM) and not the interfibrillar mitochondria (IFM), the authors set out to determine if the beneficial effects of ZP1609 were mechanistically driven by Cx43 hemichannel activity in SSM or the modulation of an as of yet undiscovered mitochondrial pathway.

In their analysis, Boengler *et al*. (2017) performed mitochondrial isolation of both SSM and IFM cardiomyocyte subsets, which differ in Cx43 immunoreactivity, but also in cellular location, respiration rate and probably ion buffering function. What is surprising about this study is the contraindicating results that support a Cx43-independent effect of ZP1609 on isolated mitochondria. The authors found that ZP1609 decreases both complex 1 and to a greater extent complex 2 respiration in SSM and IFM, with a concomitant reduction in ATP in both. These results run counter to previous observations from Cx43-knockout and gap 27 (Cx43 inhibitor)-treated mitochondria, which blocks complex 1 respiration only in SSM. In this study, it was observed that ZP1609 had no effect on mitochondrial calcium retention and ROS generation, or reductions in potassium influx, which also run counter to the effects of treatment with other Cx43 inhibitors. These mitochondrial parameters are important indicators of mitochondrial health during I/R and are proposed to contribute to the cardioprotective effects of Cx43 hemichannel inhibition. After molecular pathway analysis, the authors concluded that ZP1609 can produce beneficial cellular effects on the hypercontracture phenotype associated with cellular calcium overloading, Their results also revealed off target effects of ZP1609 on metabolically important kinases (GSK3B and Akt), which have controversial links to mitochondrial Cx43 (Rottlaender *et al*., 2012a,b) but also proven beneficial effects in I/R protection.

Although Boengler *et al*. (2017) do not directly provide evidence for ZP1609 effects on Cx43 and, in fact, argue the opposite for mitochondrial Cx43, their study highlights important questions for moving forward. Firstly, it emphasizes the need for innovative and specific tools for targeting connexins in I/R and for rigorous analysis of off-target effects of current connexin tools. Secondly, if Cx43 is present in a subset of mitochondria, a strong focus should be placed on delineating how these proteins translocate to mitochondria, how do they differ from connexins that form gap junctions, and do they directly influence mitochondrial function through channel gating or by indirect means? Thirdly, the future success of therapeutically targeting Cx hemichannels lies in understanding the molecular metabolome that passes through Cx hemichannels, given that their opening under pathological conditions can lead to cellular and potentially organelle dysregulation. Lastly, the thoughtful analysis by Boengler and collaborators underscores an important cautionary tale in targeting the complex nature of Cx43 using peptide-based therapeutics – analytical rigour and communicative carefulness are paramount to not perpetuating unintentional claims of drug selectivity, but importantly to promote clear understanding in the future use of this important class of pharmacotherapy.

Conflict of interest

The authors declare no conflicts of interest.

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