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REVIEW ARTICLE

From Erythropoietin to Its Peptide Derivatives: Smaller but Stronger

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Abstract: Erythropoietin (EPO), recognized early as a tissue protective agent, can trigger anti-inflammatory and anti-apoptotic processes to delimit injury and promote repair by binding tissue-protective receptor (TPR). However, only at a high dosage can EPO exert tissue protective effect, which may elicit severe side-effects at the meantime. Helix B surface peptide (HBSP), a 11-amnio acid sequence derived from the non-erythropoietic helix B of EPO, not only shows higher affinity to TPR but also plays a more specific and powerful role in tissue protection without erythropoietic side-effects. While it has obvious merits, the 2-min plasma half-life of HBSP restricts its application *in vivo*. Therefore, based on the amino acid sequence of HBSP, we originally designed and synthesized thioether-cyclized helix B peptide (CHBP) for an increased resistance to proteolytic degradation as well as an improved tissue protective potency, implying a brighter prospective for translational application. In this review, we will mainly discuss the development from EPO to CHBP, the merits and limitation of CHBP and the probable mechanism mediating tissue protection.

Keywords: Erythropoietin, HBSP, CHBP, ischemia-reperfusion injury, tissue protection, derivatives.

1. INTRODUCTION

Ischemic tissue injury, histologically characterized by cellular apoptosis and inflammatory infiltration within involved tissues [1], may occur in multiple organs in hospitalized patients due to such reasons as organ transplantation [2], shock [3], arterial occlusion [4] and others. Considering the high morbidity and mortality it has caused, fighting against ischemic tissue injury is still a major clinical challenge and intractable, unsolved problem worldwide. Erythropoietin (EPO), recognized early as a tissue protective agent, can trigger anti-inflammatory and anti-apoptotic processes to delimit injury and promote repair in ischemic injury models [5]. However, the relatively high dosage and severe side effects of EPO disrupted its application in the clinic. Therefore, necessary structural transformation and chemical modification of EPO is required for its translational application. Here we are willing to share the developing process from the original EPO to the newly designed and synthesized derivative of EPO--thioether-cyclized helix B peptide (CHBP). Notably, the advantage of CHBP over its predecessors and the potential for translational application will be discussed (Fig. **1**).

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2. ERYTHROPOIETIN: MORE THAN ERYTHROPOIESIS

EPO is a hematopoietic hormone produced mainly by the adult kidneys and has been routinely used in clinic for more than 20 years in the management of anemia [6]. Apart from its erythropoietic effects, EPO also exhibits powerful tissue-protective effects against acute tissue injury in a wide range of organs including kidney [7], heart [8], liver [9], and central nervous system [10]. As to the mechanisms, previous studies have demonstrated that EPO can activate multiple intracellular signalings, including mitogen activated protein kinase (MAPK), c-Jun N-terminal Kinase (c-JNK), and phosphatidylinositol 3-kinase(PI3K) signaling cascades [11], and induce the subsequent transcription of anti-apoptotic [5] and anti-oxidative genes [12]. Our study in murine model of renal injury showed that EPO protected kidneys against injury in an anti-inflammatory manner, through decreasing myeloperoxidase (MPO) positive neutrophils and suppressing the expression of pro-inflammatory cytokines and chemokines mediated by inhibiting NF-kB signaling pathway [13]. Moreover, in isolated haemoperfused porcine kidneys, it was demonstrated that EPO promoted inflammatory cell apoptosis, drove inflammatory and apoptotic cells into tubular lumens, eventually leading to inflammation clearance and renoprotection [14].

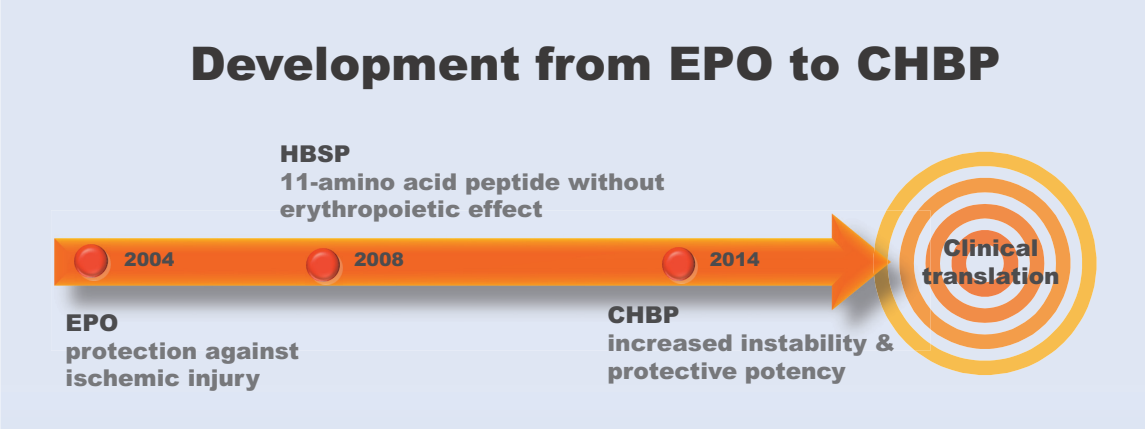

Fig. (1). The schematic picture for the timeline of development from EPO to its Derivative peptides.

3. FROM EPO TO HBSP: A SPECIFIC PROTECTIVE PEPTIDE WITHOUT ERYTHROPOIETIC EFFECT

The tissue protective effect of EPO, however, only occurs at a high dosage, which may elicit severe side-effects associated with its erythropoietic effects at the meantime, for high dosage of EPO was reported to increase the incidence of hypertension and hypercoagulation [15]. For these reasons, the tertiary structure of EPO as well as its molecular interaction with the erythropoietic receptor $(EPOR)_2$ have been identified. Interestingly, only the portions of helices A and C, as well as helix D and the loop connecting helices A and B within the dimensional structure are essential for interacting with $(EPOR)_2$ [16]. Chemical or mutational modifications of amino acid residues within these regions of EPO could abolish its binding to $(EPOR)_2$. Therefore, these modified EPOs are not erythropoietic but retain potent tissue-protective properties [17]. Thus, there ought to exists an additional receptor for EPO mediating tissue protection, which is lately defined as a heterodimer composed of EPOR and CD131, the common receptor (βcR) [18]. CD131 also forms receptor complexes with α receptor subunits specific for GM-CSF, IL-3, and IL-5 and has been termed the ''common'' receptor. In aqueous media, helix B and parts of the AB and CD loops face the aqueous medium but away from the erythropoietic binding sites, which indicates that it is helix B of EPO that mediates tissue protective effect via binding with EPOR/βcR [19].

Based on the structure of helix B of EPO, an 11-amino acid linear peptide comprising only these surface amino acids was synthesized as helix B Surface Peptide (HBSP) [20]. Abolishing the side effect of erythropoiesis, HBSP possesses an even more potent protective activity than EPO both *in vitro* and *in vivo*. Brines *et al.* in their studies showed that HBSP protected against kainic acid (KA)-induced motoneuron toxic death *in vitro* and was neuro-protective in a rat model of middle cerebral artery occlusion as well [21]. Similarly, Ueba *et al.* reported that HBSP suppressed coronary atherosclerosis in human umbilical vein endothelial cells and human monocytic THP-1 cells *in vitro* and Watanabe heritable hyperlipidemic spontaneous myocardial infarction rabbits *in vivo*, in part by inhibiting endothelial cell apoptosis through activation of Akt and in association with decreased TNF-α production and modified M1/M2 macrophage polarization in coronary atherosclerotic lesions [22]. In other studies, HBSP was reported to accelerate wound healing and modulate cognitive function in rat models [21]. The research about HBSP in our laboratory mainly focused on its protective role in renal ischemia-reperfusion injury (IRI). IRI, an inevitable pathophysiological process occurring during kidney transplantation, is closely associated with delayed graft function, acute rejection, and chronic allograft nephropathy [23]. To fight against IRI, several appreciable peptides and cell were identified and detected in our center, of those HBSP showed great promise for therapeutic application. We found in our study that HBSP played a significant protective role by ameliorating renal dysfunction and tissue damage in a murine renal IR injury model. HBSP alleviated apoptosis by inhibiting the activation of caspase-9 and caspase-3 [24]. This was in accordance with the anti-apoptotic effect of HBSP observed in cardiomyocytes suffered from oxidative stress *in vitro* and in ischemic myocardial damage *in vivo* [25]. Another interesting finding among our results was that the expression of heterodimer receptor-β common receptor (βcR)/EPOR was up-regulated by IR injury, but down-regulated by HBSP. The PI3K/Akt pathway might be involved in the negative feedback regulation of βcR/EPOR [24]. The effects of HBSP were further evaluated on the kidneys subjected to an initial IR followed by cyclosporine A(CsA) induced injuries mimicking a clinical post-transplant setting in a 2-week rat model. In this study, the tubulointerstitial damage (TID) within kidney tissue was significantly aggravated by CsA but restrained by HBSP treatment with no improvement in the IR kidneys, suggesting that HBSP attenuated CsA induced TID more effectively than IR-induced TID at 2 weeks. In addition, HBSP minimized MPO positive cells in the interstitial areas, which were increased by IR and/or CsA, suggesting an anti-inflammatory role of HBSP in IRI apart from alleviating apoptosis [26].

4. FROM HBSP TO CHBP: A CYCLIZED PEPTIDE WITH INCREASED INSTABILITY AND PROTECTIVE POTENCY

Despite its powerful tissue-protective function in various organs by inhibiting inflammation and apoptosis, it is regretful that the poor cell permeability, secondary structure insta-

bility and relatively short half-time of HBSP restricts its application *in vivo* [21]. Therefore, structurally optimized transformation of HBSP is urgently required. It is acknowledged that peptide cyclization provides an effective approach to tackle these problems [27]. Provoked by this, we applied the head-to-tail cyclization strategy to HBSP for improved stability and activity in which the peptide backbone was constrained via cyclization through main chain to main chain linkages by using thioether formation. This novel designed and synthesized peptide was named as thioether-cyclized helix B peptide (CHBP). In the following study, we have demonstrated that CHBP was significantly stable in the human plasma and exhibited a 2.5-fold longer half-life time than the corresponding linear peptide HBSP, suggesting CHBP was highly resistant to proteolytic degradation and prolonged in half-life time compared with HBSP both *in vitro* and *in vivo*. Stunningly, we also found in our research that benefiting from its stability, this long-acting CHBP could ameliorate IR injury with better outcomes than HBSP, for only one dose of CHBP exerted persistent renal protection throughout the one week reperfusion injury *in vivo*. Autophagy is demonstrated to play a renoprotective role in IR injury and is closely related to cellular apoptosis and inflammatory activation [28]. To better understand the mechanism involved in tissue protective effect of CHBP, we for the first time identified CHBP-induced autophagy in the IR-injured kidney by upregulation of the LC3-II/I ratio and increased expression of beclin-1. Furthermore, our study depicted possible signaling pathways, including mTOR regulation and AMPK activation, which link CHBP-induced autophagy with apoptosis and inflammatory responses. The activated AMPK by CHBP phosphorylated and activated tuberous sclerosis 2 (TSC2), which connected with tuberous sclerosis 1(TSC1) as a heterodimer to inhibit mammalian target of rapamycin complex 1 (mTORC1). Meanwhile, the mammalian target of rapamycin complex 2 (mTORC2)-Akt pathway was activated by CHBP and the altered mTORC1/mTORC2 equilibrium resulted in the induction of autophagy. In addition, CHBP upregulated regulatory T cell (Treg) and inhibited Th17 during renal IRI to modulate the adaptive immunity in terms of restoring the Treg/Th17 balance, which also accounted for its protective effect against tissue injury [29]. In the transport of donated organs, any approaches that prevent IRI occurring in the cold storage (CS) and reperfusion stages effectively would be of vital importance. Thus, we administrated CHBP in preservation solution and autologous blood perfusate in the isolated ischemic porcine kidneys in the following study. The results showed that the administration of CHBP during cold preservation of kidneys as well as autologous blood ameliorated IRI after hemoreperfusion with better renal blood flow, oxygenation, tubular function and tissue structure, providing confirmed experimental basis for its translational application in clinic.

5. PERSPECTIVE AND LIMITATION FOR TRANSLATIONAL APPLICATION

The research about CHBP in our center also focused on its protective effects in other kinds of injured tissues, for example, in anoxic cardiomyocytes *in vitro* and in murine acute and chronic myocardial infarction models *in vivo*. The study on the role of CHBP in acute allograft rejection is in progress as well. Though our understanding of CHBP has significantly increased, there is still plenty of work ahead of us for propelling this protective peptide from bench to clinic in the future. We have to admit that toxicological parameters of CHBP are not obtained so far, for clinical trials are indispensable before it is eventually applied. Besides this, the formulation of this new drug should be improved for oral administration or intravenous injection. In the further study, we are planning to testify on the protective effects of CHBP in primate models of acute organ injury which could better mimic diseases in human beings. We believe that this smaller but stronger peptide could facilitate the treatment for fighting against acute kidney injury in the near future.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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