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Recent Advances in KPV Peptide Delivery

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Abstract

Current research updates in last decade for α-MSH analogue, Proline-Lysine-Valine (KPV) is provided in this review. KPV, although a small molecule, is extremely hydrophilic to deliver through skin and oral delivery is challenging owing to its enzymatic degradation. Further, a clear mechanism of action for KPV has also not been understood completely. Research investigations that shed light into the mechanistic of KPV action is presented in this review. Further, application of KPV and its newly found/though derivatives in treating various indications have also been presented. Finally, challenges in further development of KPV based products have also been discussed.

Keywords: KPV, Alpha-MSH, Delivery, CKPV

Introduction

With the technological advancements, pharmaceutical research has expanded the horizon of drug delivery systems for fast, safe, and effective drug delivery. Traditional dosage forms included predominantly oral formulations such as tablets and capsules [1-3], extensive research has also been done in the area of liposomes, microparticles, nanoparticles/nanoemulsions [4-7], and also in novel route of delivery such as nose-to-brain delivery [8,9]. Further, active techniques such as iontophoresis [10], ultrasound [11], microneedles [12,13], chemical enhances [14] etc., are also being utilized to improve drug delivery.

These advanced/active systems apply to both small molecules that are synthetically derived as well as mid-to large molecules such as peptide and protein, that are biologically derived [15-20]. One such biologically derived peptide is α-MSH. α-MSH is a neurohormone with extensive immunomodulatory effects on certain cell types. It exhibits potent anti-inflammatory effects by binding to melanocortin receptors in the skin [21-23]. It is reported that the anti-inflammatory activity of α-MSH is mediated by the three terminal amino acids, Lysine-Proline-Valine (KPV). KPV lacks the entire sequence motif required for binding to the MC-Rs, but still retains almost all the anti-inflammatory capacity of the parent hormone [24-26]. This is also because peptides (like KPV) often act as hormones and relay information from one tissue through the blood to another *via* biologic

messengers [27]. The inflammatory activity of KPV is suggested to be mediated *via* inhibition of interleukin (IL)-1β. Unlike α-MSH, KPV is free from the melanotropic effects and thus does not cause any pigmentation. Additionally, it is smaller in size and more chemically stable than α-MSH. These attributes make KPV a suitable candidate for the treatment of inflammatory skin disorders. KPV has a molecular weight of 383.49 Da and an Isoelectric point (pI) of 14 and hydrophilic in nature [25,26,28].

In an investigation published by Stephen Land, the study delves deep in understanding the mechanism by which KPV, and the agonist of the dominant melanocortin receptor in airway epithelium (MC3R), suppress inflammation in immortalised human bronchial airway epithelium. Data indicated that KPV was found to suppress TNF α-evoked NFκB-driven luciferase reporter gene activity in a dose dependent manner, and this was mirrored by a decrease in IL8 release at concentrations of KPV ranging from 0.1 to 10 μg/mL, with 0.1 μg/mL of KPV resulting in IL8 secretion of ~ 400 ng/mgP and 10 μg/mL of KPV resulting in IL8 secretion of ~ 110 to 120 ng/mgP. Same concentration range of KPV was found to inhibit NFκB activity and reduction in secreted MMP-9 activity in comparison to the non-stimulated level. To further understand the mechanistic of KPV function, it was reported that KPV did not alter the proportion of cells in S-phase, however, it reversed the inhibition of cell growth caused by TNFα in conjunction activation of mTORC1, a branch point regulator of transcription and translation. Based on intracellular uptake and distribution data, it is suggested that KPV can enter the cell *via* the PEPTL1 transporter and directly interact with inflammatory signaling molecules. The anti-inflammatory activity of KPV was suggested to be independent of the melanocortin receptor signaling system and hypothesized be occurring through an intracellular mechanism. KPV reduces the duration of NFκB activation, thereby interacting with IκBα, p65RelA and Imp-α3 as critical mediators of NFκB activation and nuclear import, indicating that the predominant site of KPV accumulation is in the nucleus [29].

Recent research available on KPV

Applications of KPV in various immune-mediated inflammatory conditions such as dermatitis, bowel diseases, allergic asthma, and arthritis have been explored in the literature. This section will go into details of such studies reported in literature.

Xiao et al. has evaluated orally delivered KPV for treatment of ulcerative colitis, which is a difficult-to-treat indication owing to adverse effects and difficulty in delivering the drug to target cells. Hyaluronic Acid (HA)-functionalized polymeric nanoparticles were prepared which were then loaded with KPV. The nanoparticles observed to promote mucosal healing of wounded epithelial layers in a significant and dose-dependent manner as compared to untreated control layers when tested in Caco2-BBE monolayers. About 2-fold higher resistance was observed with functionalized nanoparticles as compared to Control at ~ 30 hrs. HA functionalized nanoparticles also demonstrated increased uptake by Colon-26 Cells and Raw 264.7 macrophages of about 1.3-fold over a period of 5 hrs, as compared to non-functionalized nanoparticles. Reduced TNF-α mRNA expression levels were reported by HA-functionalized KPV nanoparticles, with a 1.5- and 2.6- fold decrease over 24 hrs at 20 ng/mL as compared to non-functionalized nanoparticles and control, respectively. At 48 hrs, however, no significant difference was observed between

functionalized and non-functionalized nanoparticles. Further a higher tissue and cellular uptake was observed with HA-functionalized nanoparticles by CD11b+ F4/80lo colonic macrophages and CD11b+ F4/80hi macrophages by 12 hrs post-administration at 74.7% and 10.4%, respectively, in comparison to non-functionalized nanoparticles that were at 66.1% and 4.8%, respectively. This corroborated that HA functionalization improved the targeting capability of KPV nanoparticles *in vivo*. Lastly, functionalized nanoparticles in a dextran sulfate sodium (DSS)-treated ulcerative colitis mice model showed the smallest maximal loss of body weight, and thereafter exhibited the best body weight recovery, when compared to DSS control and non-functionalized nanoparticles.

Activity of KPV on Peptide Delivery

In another study, Dalmasso and group investigated the anti-inflammatory properties of KPV in intestinal inflammation for Inflammatory Bowel Diseases (IBD). KPV was added to drinking water and inflammation was assessed at the histologic level and by proinflammatory cytokine mRNA expression. This study also evaluated if the anti-inflammatory effect of KPV is PepT1-mediated (hPepT1, a di/tripeptide transporter normally expressed in the small intestine and induced in colon during IBD). It was reported that the anti-inflammatory activity is indeed PepT1 -mediated. Nanomolar concentrations of KPV inhibited the activation of NF-κB and MAP kinase inflammatory signaling pathways and reduced pro-inflammatory cytokine secretion. KPV delayed NF-κB activation and also shortened the delay of IκB-α recovery, suggesting that KPV reduced the duration of NF-κB activation. In the presence of KPV, the IL-1β–induced increase of IL-8 mRNA was also reported to be reduced significantly (by ~ 35%). This decrease of IL-1β–induced activation of NF-κB by KPV was reversed completely by the addition of Gly-Leu, confirming that KPV's activity is mediated by hPepT1. Assessment of KPV's anti-inflammatory effect in Jurkat cells was substantiated by real-time RT-PCR. After 6 hrs of stimulation, TNF-α induced an approximately 5-fold increase of IL-8 mRNA, which was reduced significantly in the presence of KPV. In conclusion, orally delivered KPV seem to reduce the intestinal inflammation or colitis condition, thus providing a promising therapeutic candidate for IBD indication [25].

A research study presented by Singh et al. showed that C-terminal amino acids of α-MSH are requisite for its antibacterial activity against *Staphylococcus aureus* [32]. KPV was studied along with other analogs of α-MSH for this investigation. It was reported that KPV which is α-MSH (11-13) efficiently killed of both methicillin-sensitive and -resistant *Staphylococcus aureus* cells in dose dependent manner. 1 μM of KPV killed 95% ± 0.97%, 96.2% ± 0.46%, and 97.3% ± 0.98% of the cells, respectively, in 2 h (P<0.001), whereas 10 pM of KPV showed a 35% ± 9.98%, 29% ± 0.65%, and 19% ± 6.18% bacterial killing, respectively (P<0.001) in comparison to parent peptide and other analogs. KPV's antibacterial efficiency was comparable to that of the entire α-MSH. In addition to bactericidal potency, to determine the rate of killing by KPV, Staphylococcus cells were incubated with KPV for 0 min to 120 min [22]. An extremely rapid bactericidal activity was observed with ~90% of cells   were killed within 15 min of incubation. The antimicrobial activity of KPV was not affected by the presence of NaCl or even divalent cations such as Ca2+ and Mg2+. Similar to parent peptide, KPV also depolarized and permeabilized Staphylococcus cells at micromolar concentrations.

A derivative of KPV, (CKPV)2, (Ac-Cys-Lys-Pro-Val-NH2)2 is synthesized by inserting a Cys-Cys linker between the two units of KPV. Historical literature has shown excellent anti-candidacidal and anti-inflammatory effects, (CKPV)2 demonstrated a fungistatic rate of Candida albicans at up to 50% and 90% at 3 X 10-8 M and 10-6 M, respectively. Further, in rats, (CKPV)2 at 2 mg/kg showed the strongest inhibition against vaginal Candida albicans, with survival of Candida albicans dropping to 12.0% at the 11th day of the treatment and to 0% at 18th day of treatment, Miconazole on the other hand, at 0.5 mg/kg showed 44.7% drop in survival rate. This study also proved that (CKPV)2 also induced its anti-inflammatory effect through induction of macrophages M1 to M2 polarization. (CKPV)2 also directly inhibited phagocytosis ability of primary cultured macrophages, whereas promoting cAMP production *via* activating melanocortin receptor-1(MC1R) in macrophages. These results strongly suggest (CKPV)2 activity in fungal and inflammatory conditions for future medicines.

Schaible and group reported that a single administration of tripeptide α-MSH (11–13) or KPV attenuates brain damage by reduced inflammation and apoptosis after experimental traumatic brain injury in mice. Male C57Bl/6N mice were used for this study. KPV was dissolved in normal saline (0.9% NaCl) and injected in mice intraperitoneally 30 min after trauma. Results showed that KPV solution significancy reduced secondary brain lesion expansion possibly by reducing apoptotic cell damage, and thus offering neuroprotection. This neuroprotection was hypothesized to be mediated not only by KPV's immune-modulatory action but also by its anti-apoptotic effect. Further, a reduced secondary brain damage in KPV treated mice is reasoned to be caused at least in part by a reduction of apoptotic cell death. Based on the results obtained, it was suggested that the neuroprotective effect of KPV after traumatic brain injury might be in part caused by anti-apoptotic pathways, specifically *via* MC4R-mediated activation.

In order to improve stability *in vivo* against proteolytic enzymes, pharmacokinetics, and oral bioavailability, Songok and team performed structural modification of the tripeptide KPV by reductive "glycoalkylation" of the lysine residue. Pronase, a commercially available cocktail of enzymes that is been used routinely to digest proteins to their constituent amino acids was mixed with KPV and glycoalkylated KPV. Unmodified KPV degraded to its three constituent amino acids within 24 hrs, whereas α-glycoalkylated tripeptide was completely stable under the conditions of the pronase experiment. This indicates a successful modification of KPV to improve its chemical degradation without impacting its biological activity.

Pawar et al. evaluated the delivery of KPV with microneedles and iontophoresis combination across human skin, and compared with passive diffusion, microneedles alone and iontophoresis alone. KPV permeation *via* passive diffusion was below the limit of detection. With microneedles treatment alone, KPV permeation was increased to 4.4 μg/cm2/h whereas iontophoresis alone and combination increased the permeation by 8 and 35-fold, respectively, when compared to microneedles alone. The low permeation with microneedles alone was attributed to the fact that despite the assistance from microneedles, it is still a passive delivery unlike iontophoresis which is an active delivery technique. The theory of combining these two techniques to increase skin permeation for an extremely water-soluble drugs like KPV was already tested by the same group of researchers in their other publication. Further, a stability indicating analytical method was also

developed by the same group of scientists to determine KPV levels in skin homogenates. The calibration curve was linear with a correlation coefficient (r) of 0.9999. Relative standard deviation values of accuracy and precision experiments were <2. The LOD and LOQ were reported to be 0.01 and 0.25 µg/mL, respectively. Additionally, the delivery of KPV can be controlled by optimizing various parameters such as current density, duration of applied current strength, influence of microneedles pretreatment, and KPV concentration. Thus, the aim of the current study was to investigate the feasibility and optimize the transdermal delivery of KPV.

Conclusion

A review of research done in last decade on the α-MSH analogue, KPV has been presented. Based on the limited literature available in last decade, it indicates that the mechanism of action for KPV is still not fully understood. Further, the hydrophilicity of molecule may have discouraged the scientific community from exploring it for its applications in various disease indications. Literature showed that further modification or functionalization of KPV such as addition of cysteine group or glycoalkylation can significantly improve its pharmacological activity and/or chemical stability. It would be interesting to see more literature in future with various chemical modifications to KPV with help of Structural Activity Relationship (SAR) to further improve its anti-inflammatory activity as well as physical and chemical stability over shelf life. Further, oral route can also be explored for KPV for chronic systemic inflammatory conditions, such as Inflammatory Bowel Diseases (IBD), rheumatoid arthritis, hashimoto thyroiditis, multiple sclerosis, systemic lupus erythematosus, etc. various advanced drug delivery systems such as liquid filled capsules, film/enteric coated tablets, osmotic delivery system, etc. would also open doors for delivery of KPV with a higher systemic bioavailability. With current line of treatment being steroids for most of the chronic, inflammatory conditions, a myriad of adverse effects come into play. KPV could potentially be an alternate option with no steroid-like side effects but with same anti-inflammatory effect, especially for long term treatment.

References

1. Alqahtani MS, Kazi M, Alsenaidy MA, Ahmad MZ (2021) Advances in oral drug delivery. Front Pharmacol 12: 1-21.
2. Homayun B, Lin X, Choi HJ (2019) Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. Pharmaceutics 11: 129.
3. Pawar K, Render D, Rangari V, Lee Y, Babu RJ (2018) Evaluation of non-crystalline cellulose as a novel excipient in solid dose products. Drug Dev Ind Pharm 44: 1512-1519.
4. Santos AMD, Carvalho SG, Meneguin AB, Sábio RM, Gremião MPD, et al. (2021) Oral delivery of micro/nanoparticulate systems based on natural polysaccharides for intestinal diseases therapy: Challenges, advances and future perspectives. J Control Release 334: 353-366.
5. Kai T, Jiqian W, Peng Z, Chengdong W, Hai X, et al. (2011) Self-Assembly of Short Aβ(16−22) Peptides: Effect of Terminal Capping and the Role of Electrostatic Interaction. Langmuir. 27: 2723-2730.
6. Pawar KR, Babu RJ (2014) Lipid materials for topical and transdermal delivery of nanoemulsions. Crit Rev Ther Drug Carr Syst 31: 429-458.
7. Pawar KR, Babu JR (2010) Polymeric and lipid-based materials for topical nanoparticle delivery systems. Crit Rev Ther Drug Carr Syst 27: 419-459.
8. Babu RJ, Dayal PP, Pawar K, Singh M (2011) Nose-to-brain transport of melatonin from polymer gel suspensions: A microdialysis study in rats. J Drug Target 19: 731-740.
9. Pires PC, Fazendeiro AC, Rodrigues M, Alves G, Santos AO (2021) Nose-to-brain delivery of phenytoin and its hydrophilic prodrug fosphenytoin combined in a microemulsion - formulation development and in vivo pharmacokinetics. Eur J Pharm Sci 164: 1-10.
10. Waghule T, Singhavi G, Dubey SK, Pandely MM, Gupta G, et al. (2019) Microneedles: A smart approach and increasing potential for transdermal drug delivery system. Biomed Pharmacother 109: 1249-1258.
11. Ng KW (2018) Penetration enhancement of topical formulations. Pharmaceutics 10: 51.
12. Saepang K, Li SK, Chantasart D (2021) Passive and iontophoretic transport of pramipexole dihydrochloride across human skin microchannels created by microneedles in vitro. Int J Pharm 609: 1-13.
13. Lin WQ, Cormier M, Samiee A, Griffin A, Johnson B, et al (2001). Transdermal delivery of antisense oligonucleotides with microprojection patch (Macroflux (R)) technology. Pharm Res 18: 1789–1793.
14. Henry S, McAllister DV, Allen MG, Prausnitz MR (1998). Microfabricated microneedles: A novel approach to transdermal drug delivery. J Pharm Sci 87: 922-925.
15. Lanza JS, Vucen S, Flynn O, Donadei A, Cojean S, et al. (2020) A TLR9-adjuvanted vaccine formulated into dissolvable microneedle patches or cationic liposomes protects against leishmaniasis after skin or subcutaneous immunization. Int J Pharm 586: 1-14.
16. Donadei A, Kraan H, Ophorst O, Flynn O, O'Mahony C, et al. (2019) Skin delivery of trivalent Sabin inactivated poliovirus vaccine using dissolvable microneedle patches induces neutralizing antibodies. J Control Release 311-312: 96-103.
17. Luger A, Scholzen TE, Brzoska T, Böhm M (2003) New insights into the functions of alpha-MSH and related peptides in the immune system. Ann N Y Acad Sci 994: 133-40.
18. Barcellini W, Colombo G, La Maestra L, Clerici G, Garofalo L, et al. (2000) Alpha-melanocyte-stimulating hormone peptides inhibit HIV-1 expression in chronically infected promonocytic U1 cells and in acutely infected monocytes. J Leukoc 68: 693-699.
19. Van Klaveren RJ, Morshuis WJ, Lacquet LK, Cox AL, Festen Jet al. (1992) Congenital bronchial atresia with regional emphysema associated with pectus excavatum. Thorax 47: 1082-3.
20. Xiao B, Xu Z, Viennois E, Zhang Y, Zhang Z, et al. (2017) Orally targeted delivery of tripeptide KPV via hyaluronic acid-functionalized nanoparticles efficiently alleviates ulcerative colitis. Mol Ther 25: 1628-1640.
21. Dalmasso G, Hisamuddin LC, Nguyen HTT, Yan Y, Sitaraman S, et al. (2008) PepT1-mediated tripeptide KPV uptake reduces intestinal inflammation. Gastroenterology 134: 166-178.