Enhanced anti-sarcopenic activity of perindopril erbumine-entrapped ultradeformable liposomes via skin delivery in muscle atrophy mouse model

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ABSTRACT

Sarcopenia is a pertinent challenge in the super-aged societies causing reduced functional performance, poor quality of life and increased morbidity. The aim of this study is to prepare perindopril erbumine-entrapped ultradeformable liposomes (PER-UDLs) with enhanced skin permeation and to investigate the anti-sarcopenic activity of optimized PER-UDLs in lipopolysaccharide (LPS)-induced muscle atrophy mouse model. PER-UDLs were prepared by thin-film hydration and extrusion technique using egg yolk phosphatidylcholine as a bilayer forming lipid component and Tween 80 or sodium deoxycholate as an edge activator. The deformability of PER-UDLs was determined and compared with that of conventional liposomes by penetrating formulations through a filter device at a constant pressure. The in vitro skin permeation of PER-UDLs was assessed using Franz diffusion cell. In vivo anti-sarcopenic effects of PER-UDLs were investigated in lipopolysaccharide-induced muscle atrophy mouse model. Owing to the smallest particle size (75.0 nm) and the highest deformability (54.2) and entrapment efficiency (35.7%), PER-UDLs with EPC to Tween 80 ratio of 8:2 was selected as the optimized formulation. The optimized PER-UDLs showed significantly higher cumulative amount of drug permeated and permeation rate across the rat skin compared to PER solution (485.7 vs. 50.1 μg and 13.4 vs. 2.3 μg/cm^2/h, respectively). Topically applied PER-UDLs successfully alleviated the LPS-induced sarcopenia in mice by improving body weight changes, grip strength and muscle weight. Furthermore, PER-UDLs reduced the shrinkage of muscle fibers as demonstrated by higher cross-sectional area than PER solution. PER-UDLs also increased the expression of myosin heavy chain (MHC) protein and reduced the expression of muscle atrophy F-box (Atrogin-1) and muscle ring-finger protein-1 (MuRF1), thereby improving muscles atrophy. In conclusion, these results indicate that PER-UDLs have a great potential as sarcopenia therapeutics.

INTRODUCTION

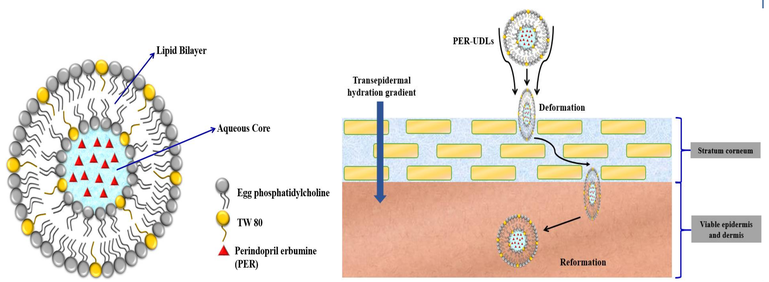
Ultradeformable liposomes (UDLs)

- **UDLs** are liposomal carriers consisting of **phospholipids** and **edge activators.**
- Edge activators destabilize the lipid bilayers and impart **high deformability** to the vesicle.
- UDLs have **better permeation through the skin** compared to the conventional liposomes.

Perindopril Erbumine (PER)

- **PER** is an ACE inhibitor mainly used to treat hypertention and also known to have anti-sarcopenic effect via inhibition of angiotensin II.
- PER oral tablets have several limitations including first-pass effect, difficulty of swallowing in elderly patients and risk of side effects.
- **Skin delivery of PER** using **UDLs** could be a promising alternative to sarcopenia therapeutics.

Schematic illustration of UDLs & skin permeation mechanism

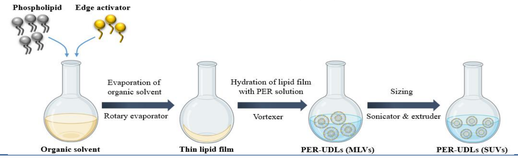


METHODS

Preparation of PER-loaded Ultra Deformable liposomes (PER-UDLs)

Formulation	EPC	SDC	TW80	CH	PER
PER-UDLs-S1	9	1			10
PER-UDLs-S2	8	2	-	-	10
PER-UDLs-S3	7	3	-	-	10
PER-UDLs-T1	9		1		10
PER-UDLs-T2	8	-	2	-	10
PER-UDLs-T3	7	-	3	-	10
PER-CLs	8	-	-	2	10

EPC: egg phosphatidylcholine, SDC: sodium deoxycholate, TW80: Tween 80, CH: cholesterol, PER: perindopril erbumine

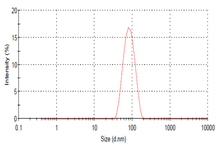


RESULTS

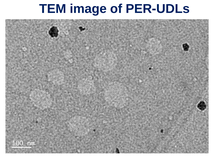
Physicochemical properties & deformability of PER-UDLs

Formulation	Size (nm)	PDI	Zeta Potential (mv)	Entrapment efficiency (%)	Deformability index(J)
PER-UDLs-S1	80.4 ± 0.9	0.051 ± 0.021	-15.1 ± 0.8	20.0 ± 0.8	43.3 ± 3.6
PER-UDLs-S2	85.3 ± 0.3	0.055 ± 0.028	-20.0 ± 1.0	24.1 ± 1.8	52.1 ± 4.0
PER-UDLs-S3	79.5 ± 7.6	0.224 ± 0.047	-13.8 ± 5.0	25.5 ± 6.9	47.5 ± 3.9
PER-UDLs-T1	82.0 ± 2.3	0.053 ± 0.011	-8.8 ± 0.5	20.5 ± 2.0	39.3 ± 1.7
PER-UDLs-T2	**75.0 ± 1.1**	**0.072 ± 0.007**	**-10.8 ± 1.9**	**35.7 ± 4.2**	**54.2 ± 2.3**
PER-UDLs-T3	79.4 ± 0.9	0.095 ± 0.024	-8.5 ± 1.5	33.2 ± 2.3	49.0 ± 6.8
PER-CLs	126.9 ± 12.4	0.095 ± 0.020	-3.3 ± 0.8	25.7 ± 6.8	26.1 ± 4.2

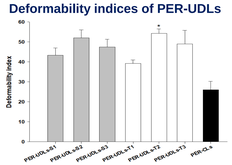
Size Distribution of UDLs



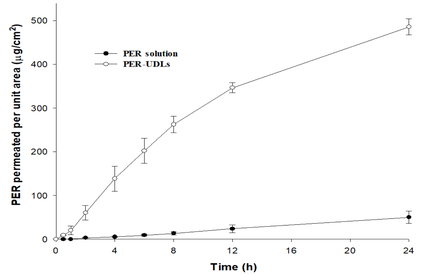
TEM image of PER-UDLs



Deformability indices of PER-UDLs



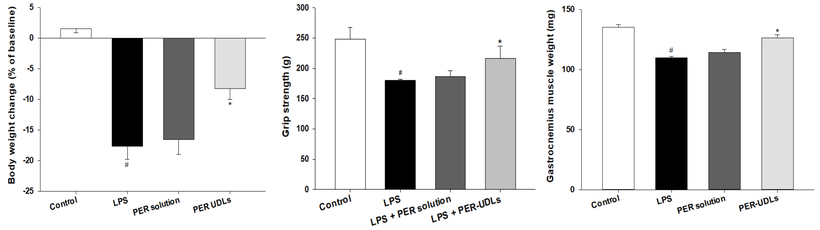
In vitro skin permeation of PER-UDLs



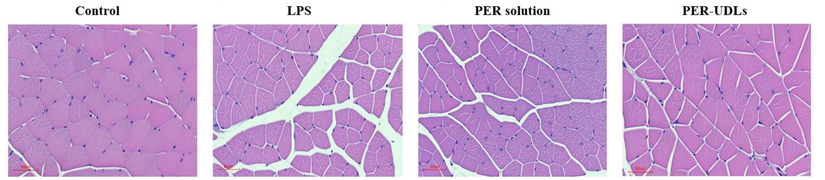
Formulation	Cumulative amount of PER permeated in 24 h (Q_{24}, μg)	Permeation flux (J, μg/cm²/h)	Permeability coefficient (K_p, cm/h)	Enhancement ratio
PER solution	50.1 ± 14.4	2.3 ± 0.6	1.5×10^{-3}	-
PER-UDLs	485.7 ± 18.6*	13.4 ± 0.8*	8.9×10^{-3}*	5.9

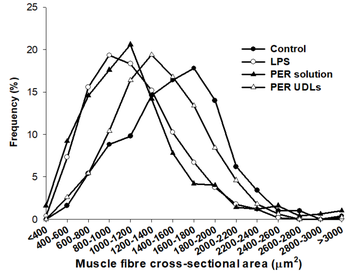
In vivo anti-sarcopenic effect of PER-UDLs in LPS-induced muscle atrophy model

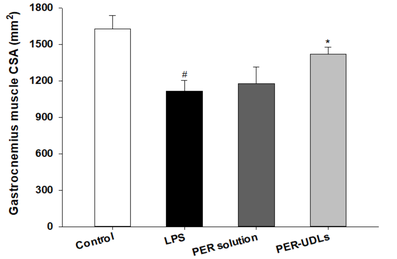
Effects of PER-UDLs on the body weight change, grip strength and gastrocnemius muscle weight



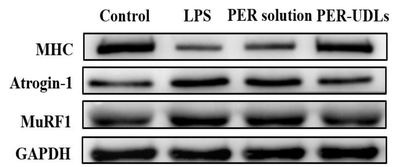
Histopathological evaluation of gastrocnemius muscles

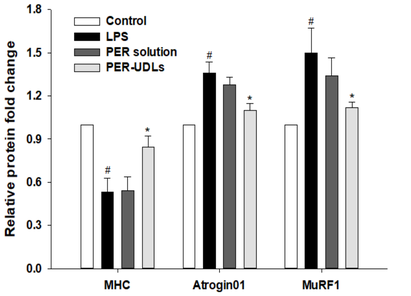






Effects of PER-UDLs on the expression of muscle atrophy-related proteins





CONCLUSION

Collectively, these results suggest that PER-UDLs could be a promising transdermal delivery system for potential sarcopenia therapy.