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ABSTRACTS

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The urinary metabolite Ac-Tβ$_{1-14}$ in huvec cells and rats treated with thymosin β4: A potential biomarker for doping tests

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Abstract

Thymosin β4 (**Tβ4**) was reported to exert various beneficial bioactivites such as tissue repair and regeneration, anti-inflmmation, and reduced scar formation, and it is listed on the prohibited substnaces by the World Anti-Doping Agency. No metabolism studies of **Tβ4** were not reported yet. Previously, our lab reported that total 13 metabolites were found by using multiple enzymes, and 6 metabolites (Ac-Tβ$_{31-43}$, Ac-Tβ$_{1-11}$, Ac-Tβ$_{17-43}$, Ac-Tβ$_{1-14}$, Ac-Tβ$_{1-15}$, and β$_{1-17}$) were confirmed by comparing with the synthetic standards.

The study was aimed at identifying new metabolites of **Tβ4** in biological systems such as leucine aminopeptidase, human kidney microsomes, huvec cells and rats to develope biomarkers for detecting doping in sports.

A method for detecting and quantifying Ac-Tβ$_{1-14}$ was developed and validated using Q-Exactive orbitrap mass spectrometry. LOD and LOQ of the Ac-Tβ$_{1-14}$ were 0.19 and 0.58 ng/ml and showed a good linearity (r^2=0.9998). This work was conducted in in vitro pure enzyme and microsome systems, cultured huvec cells, and rats after administration of parent protein. Among 6 metabolites above, Ac-Tβ$_{1-14}$ was found in huvec cells exposed to 10 μg/ml and in urine of rats intraperitoneally treated with 20 mg/kg **Tβ4.**

As a result, Ac-Tβ$_{1-14}$, the metabolite of **Tβ4**, was detected in all in vitro biological systems including pure enzyme and microsome systems, huvec cells, and rat urine samples. In addition, the metabolite Ac-Tβ$_{1-14}$ was quantitatively determined to 48 hr in rats, with the highest concentration occurred between 0-6 hr. Ac-Tβ$_{1-14}$ was not detected in non-treated control groups including human blank urine.

This results suggest that Ac-Tβ$_{1\text{-}14}$ in urine is a potential biomarker for screening the parent Tβ4 in doping tests.