An innovative biomimetic sensing platform as a promising nano-device for gonadorelin detection

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Ongoing improvements in the pharmaceutical industry have expanded the list of potential doping agents regulated and annually reviewed by the World Anti-Doping Agency (WADA) [1].
Low molecular weight peptide hormones (<2000 Da), holding a well-defined structural characteristic, represent a new frontier in antidoping research as these peptides (e.g. gonadorelin, buserelin, deslorelin, leuproprelin etc.) have been included in the S2 section of the WADA's List of Prohibited Substances [2, 3].
In this framework, we focused our attention on gonadorelin misuse in sports competitions.
Gonadorelin is a synthetic decapeptide (peptide sequence: pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, MW 1182.33 Da) that has the same chemical structure as the endogenous neuro-peptide gonadotropin-releasing hormone (GnRH) [4]. It is available as clinical and veterinary drugs (e.g. for the treatment of hypogonadism, cancer, etc.) and is improperly used by male athletes to enhance their physicals performances by stimulating the pulsatile endogenous secretion of testosterone in the bloodstream via the hypothalamic-pituitary-gonadal (HPG) axis, eventually with impact on the athlete's biological passport.
Our study aimed to develop an efficient and selective molecularly imprinted polymer (MIP)-based assay to detect gonadorelin levels in biological fluids, such as urine and plasma. The process of molecular imprinting involves the synthesis of a 3-D poly(norepinephrine) matrix with binding sites complementary in shape, size, and functional groups to the template molecules [5,6]. The interaction between gonadorelin and "tailor-made" synthetic biopolymer was preliminarily characterized by a surface plasmon resonance (SPR) sensing platform. Afterward, a competitive inhibition biomimetic assay was designed employing a biotinylated gonadorelin as competitor molecule. This type of assay is appropriate for the detection of small molecules which lack multiple epitopes. Encouraging results were recorded for gonadorelin in buffer and synthetic urine samples by using a simple biotin-streptavidin signal amplification strategy. Moreover, we intend to provide a strategy to detect gonadorelin which can be easily miniaturized, by tuning different amplification strategy embedded with nano-MIPs, to set-up a point-of-care (POC) sensing device for in-situ athletes' monitoring.

References

[1] Scarano, S., Ermini, M.L., Spiriti, M.M., Mascini, M., Bogani, P., Minunni, M., Anal. Chem. 16, 6245-53 (2011).
[2] Botrè, F., de la Torre, X., Mazzarino, M., Bioanalysis. 11, 1129-32 (2016).
[3] https://www.wada-ama.org
[4] Helm, V.J., Müller, B.W. Pharm. Res. 7, 1253-1256 (1990).
[5] Palladino, P., Minunni, M., Scarano, S. Biosens. Bioelectron. 106, 93-98 (2018).
[6] Baldoneschi, V., Palladino, P., Banchini, M., Minunni, M., Scarano, S. Biosens. Bioelectron. 157,112161 (2020).