International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 8, Issue 1, pp: (250-254), Month: April 2020 - September 2020, Available at: www.researchpublish.com

20-Hydroxyecdysone's Cyclodextrin Complex: A Novel Anabolic Phytosteroid (APS)?

Victor Chiruta[1]

[1] Faculty of Health Science, University of South Australia, Adelaide, Australia

***Abstract*: 20-Hydroxyecdysone (20-HE) is currently undergoing a Phase 2 Clinical Trial for the treatment of sarcopenia. 20-HE is also currently being investigated by the World Anti-Doping Agency (WADA) as an anabolic agent. There are promising in vitro and in vivo results for 20-HE's anabolic effect on muscles. However, 20-HE is molecularly a steroid, thus having relatively poor water solubility and poor human oral bioavailability. Cyclodextrins (CDs) are being used in pharmaceutics to improve the water solubility and oral bioavailability of lipophilic compounds. This paper explores 20-HE's pharmacodynamics and pharmacokinetics, with a particular interest on how the 20-HE-CD complex can improve the oral bioavailability of 20-HE. Consequently, making 20-HE-CD a novel anabolic phytosteroid (APS).**

***Keywords:* 20-hydroxyecdysone, ecdysteroid, cyclodextrin, phytosteroid.**

I. INTRODUCTION

20-HE is an ecdysteroid primarily isolated from the plant *Cyanotis arachnoidea*[1], but also present in spinach[2] and quinoa[3]. In the 1980s, ecdysteroids were suspected to be used by Russian weightlifters in the Olympics[4]. This led to a study in 2000, comparing the anabolic efficacy of ecdysteroids to a group of anabolic androgenic steroids (AAS) known as steranabols[5]. Following the positive results, in 2015, 20-HE was compared for anabolic efficacy *in vitro* and *in vivo* with a range of endogenous and exogenous anabolic compounds[4]. 20-HE had greater anabolic effects on muscle than the compared compounds.

In 2015, a non-clinical study found that 20-HE prevented catabolic expression in cartilage[6]. In 2016, another non-clinical study discovered that 20-HE combats muscle atrophy[7]. In 2016, WADA undertook an investigation to potentially prohibit this compound under the schedule of 'S2.1 Other Anabolic Agents'[8].

20-HE's previous use in the olympics, its promising non-clinical results, and the WADA investigation into its use as an anabolic agent gives the rationale for the clinical trials of 20-HE, for the treatment of muscular degenerative diseases[9]. 20-HE deserves examination as a potential APS.

II. PHARMACODYNAMICS

A. Chemical Structure

20-HE **TP**

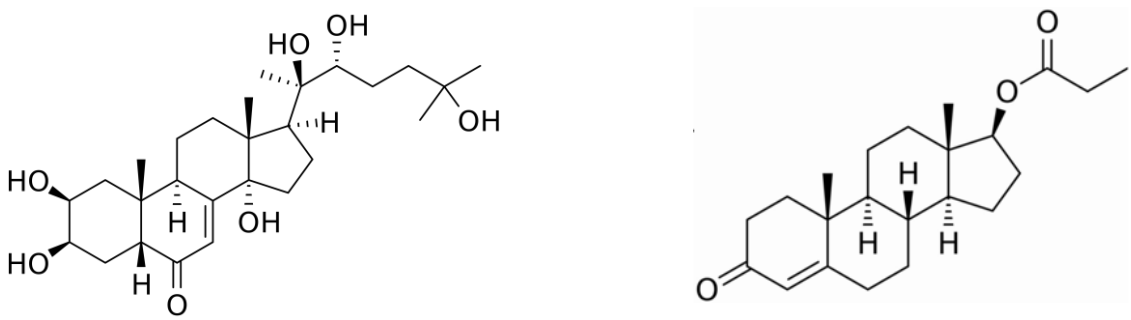


Fig. 1. Structure of 20-HE and testosterone propionate (TP). 20-HE is a structural analogue of TP.

B. Binding Affinity

20-HE has affinity with estrogen receptor-β (ER-β)[4]. ER-β is anabolic and inhibits estrogen-related tissue growth[10]. ER-β has been described as the opposite of ER-α. ER-β elicits its anabolic effects by increasing the expression of MyoD, PCNA (proliferating cell nuclear antigen), myosatellite cells, PAX7, embryonic MHC (myostatin heavy-chain), and IGF-1R (insulin-like growth factor-1 receptor)[11][12].

C. Efficacy

- MyoD is a protein of the myogenic regulatory factors involved in the differentiation of muscle cells[13].
- PCNA is a processivity factor for DNA polymerase, recruiting proteins in DNA repair[14].
- Myosatellite cells are multipotent cells found in mature muscle[15]. They are the precursors for skeletal muscle fibres by proliferating and differentiating into myoblasts[16].
- PAX7 is a transcription factor gene expressed in skeletal muscle precursor cells[17].
- Embryonic MHC is a muscle motor protein that regulates muscle function[18].
- IGF-1R is the receptor for IGF-1. IGF-1 signals the PI3K/Akt pathway, an intracellular signalling pathway in regulating cell cycle[19]. This pathway regulates quiescence (a type of cell growth fraction), proliferation, and cellular longevity[20].
- ER-β has anti-proliferative effects in reproductive tissue[10]. This suggests it may possibly help combat gynaecomastia and may assist with endometriosis.

III. PHARMACOKINETICS

A. Absorption/Administration

20-HE is a lipophilic steroid, facing poor absorption issues in the gut. Absorption in the gastrointestinal (GI) tract is contingent on a molecules water-solubility in the GI juices and overall bioavailability. The solubility in water of 20-HE is very low at 0.084mg/mL[21]. This will significantly effect absorption, thus 20-HE has poor oral bioavailability. Hence, we see bodybuilders administering very large doses of 1000mg of 20-HE per day[4].

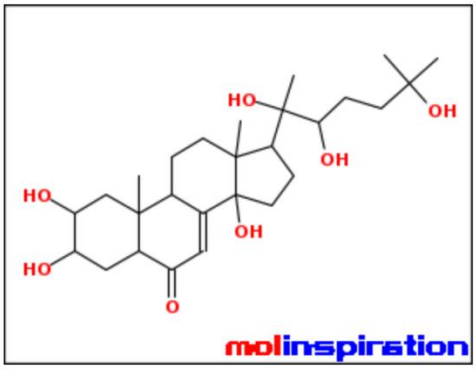
B. Metabolism

20-HE has an elimination half-life of nine hours in humans[22].

IV. *IN SILICO* MODELLING OF 20-HE

The *in silico* software used to calculate 20-HE's bioactivity in comparison with TP is called Molinspiration. Molinspiration is an advanced molecular structure bioactivity predictor for the application of modern cheminformatic techniques. Molinspiration is used to extrapolate in silico data for drug discovery.

Previously established, 20-HE is a ligand for ER-β. TP being an AAS, is a ligand for AR. Both ER-β and AR are subtypes of nuclear receptors. With Molinspiration, a direct comparison of 20-HE's and TP's nuclear receptor ligand bioactivity is possible. 20-HE has a nuclear receptor ligand bioactivity of 0.92 (Fig. 2), TP of 0.87 (Fig. 3). 20-HE (0.92) has approximately 5% more binding affinity compared to TP (0.87) for the respective nuclear receptor.



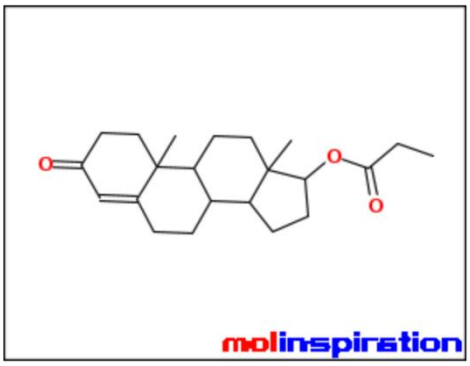
Molinspiration bioactivity score v2018.03

GPCR ligand	0.16
Ion channel modulator	0.17
Kinase inhibitor	-0.32
Nuclear receptor ligand	0.92
Protease inhibitor	0.32
Enzyme inhibitor	0.68

Get data as text (for copy / paste).

Get 3D geometry BETA

Fig. 2. Molinspiration bioactivity scores for 20-HE



Molinspiration bioactivity score v2018.03

GPCR ligand	-0.07
Ion channel modulator	-0.29
Kinase inhibitor	-0.97
Nuclear receptor ligand	0.87
Protease inhibitor	-0.10
Enzyme inhibitor	0.52

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Fig. 3. Molinspiration bioactivity scores for TP

V. *IN VITRO* AND *IN VIVO* 20-HE COMPARISONS

In Fig. 4, 20-HE is more anabolic (measured by hypertrophy) than DHT (dihydrotestosterone) and IGF-1, *in vitro*. In Fig. 4, 20-HE is more anabolic (measured by hypertrophy) than the AAS Methandienone (Dianabol), the AAS Estradienedione (Trenbolox), and the SARM (selective androgen receptor modulator) S-1, *in vivo*.

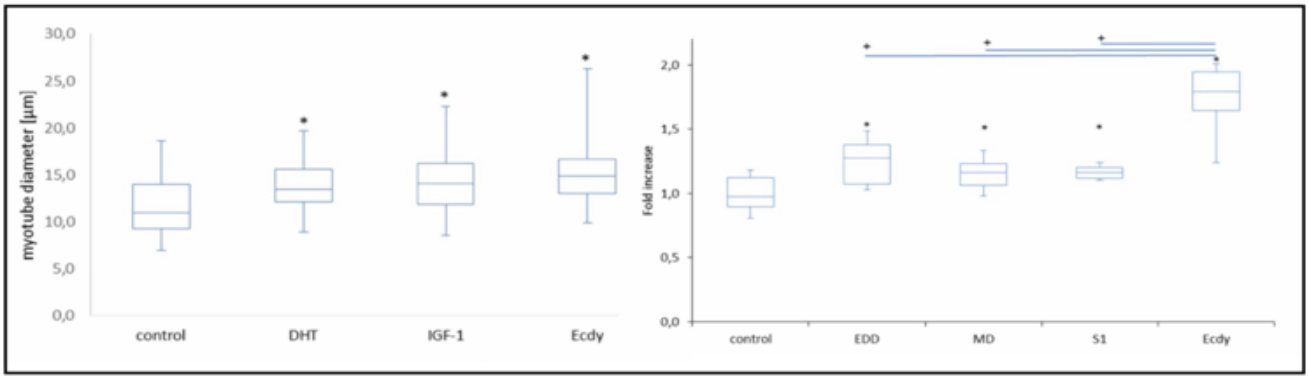

Fig. 4. On the left, 20-HE (Ecdy) vs endogenous anabolics in vitro. On the right, 20- HE (Ecdy) vs AAS and a SARM *in vivo*[4].

In Fig. 5, ecdysterone is a synonym of 20-HE. 20-HE *in vivo* has equal hypertrophic potential as the steranabol Methandriol (Methylandrostenediol). The ecdysteroid turkesterone (11α-hydroxyecdysone) was more hypertrophic than the steranabols Methandriol and Nerobol (Methandrostenolone).

Compound	Puberal (210 – 220 g)		Impuberal (70 – 80 g)			
			intact		castrated	
	weight gain, mg/(g day)	effect, % of control	weight gain, mg/(g day)	effect, % of control	weight gain, mg/(g day)	effect, % of control
Control	5.2 + 0.6	–	15.3 + 1.5	–	12.5 + 1.1	–
Ecdysterone (I)	7.9 + 0.8*	51.9	27.9 + 2.7*	82.4	18.1 + 1.4*	44.8
Viticosterone E (II)	7.3 + 0.6*	40.4	24.6 + 2.5*	60.8	16.2 + 1.1*	29.6
Ecdysterone triacetate (III)	7.1 + 0.5*	36.5	25.2 + 1.7*	64.7	15.9 + 1.1*	27.2
Ecdysterone tetraacetate (IV)	6.8 + 0.4	30.8	23.9 + 2.3*	56.2	15.2 + 1.4	21.6
2-Deoxyecdysterone (V)	6.3 + 0.5	21.2	20.7 + 1.1*	35.3	14.9 + 1.1	19.2
α-Ecdysone (VI)	6.2 + 0.5	19.2	19.5 + 1.2*	27.4	14.1 + 1.3	12.8
2-Deoxy-α-ecdysone (VII)	6.1 + 0.5	17.3	18.9 + 0.6*	23.5	14.0 + 1.3	12.0
2-Deoxy-α-ecdysone-22-*o*-acetate (VIII)	5.9 + 0.3	13.5	18.2 + 0.9	18.9	13.6 + 1.6	8.8
Sileneoside A (IX)	8.1 + 0.6*	55.8	28.8 + 2.4*	88.2	18.7 + 1.2*	49.6
Sileneoside C (X)	6.3 + 0.7	21.2	21.5 + 1.7*	40.5	14.9 + 1.6	19.2
Turkesterone (XI)	8.5 + 0.6*	63.5	33.9 + 3.0*	121.6	20.8 + 2.8*	66.4
Turkesterone triacetate (XII)	8.3 + 0.6*	59.6	31.5 + 2.4*	105.9	19.2 + 2.2*	53.6
24(28)-Dehydromakisterone A (XIII)	7.8 + 0.8*	50.0	26.8 + 2.8*	75.2	17.5 + 1.4	40.0
Integristerone A (XIV)	6.5 + 0.5	25.0	21.1 + 2.1*	37.9	15.3 + 1.9	22.4
Methylandrostenediol	7.9 + 0.5*	51.9	27.1 + 2.5*	77.1	23.0 + 2.9*	84.0
Nerobol	8.2 + 0.5*	57.7	32.7 + 3.7*	113.7	27.8 + 3.4*	122.4

* $p < 0.05$ relative to control.

Fig. 5. Anabolic efficacy of ecdysteroids vs steranabols *in vivo*[5].

VI. CLINICAL TRIALS

In 2017, Phase 1 Clinical Trial for 20-HE was conducted with the European Medicines Agency[9]. In 2018, the trial moved into Phase 2, currently still underway. The clinical trial is in the Musculoskeletal and Connective Tissue Disorders category for sarcopenia.

VII. CYCLODEXTRIN COMPLEXES FOR IMPROVED BIOAVAILABILITY

Previously discussed, bioavailability is co-dependant on GI absorption. Absorption in the GI tract is contingent on a molecules water-solubility in the GI juices. The solubility of 20-HE is very low at 0.084mg/mL in water[21]. This will negatively effect absorption. 20-HE-β-CD has a water solubility of 8.87mg/mL. This is 105 fold the water solubility of 20-HE. 20-HE-2(β-CD) has a water solubility of 9.31mg/mL in water. This is 110 fold the water solubility of 20-HE. CD complexes are an effective way to increase the water-solubility of 20-HE, thus absorption.

VIII. CONCLUSION

20-HE is a powerful anabolic compound. Its investigation by WADA and its clinical trial for its therapeutic use to treat sarcopenia attest to this. Unfortunately, 20-HE has very poor bioavailability. A method of increasing bioavailability is by the use of CD complexes.

ACKNOWLEDGEMENTS

Thank you to Robert Renshaw for spurring my interests into CDs and for the facilities of Mezl Pty Ltd who allowed me to conduct research on delivery systems to improve the oral bioavailability of lipophilic phytochemicals.

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