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The effect of esterases on 17 α -hydroxyprogesterone caproate

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Abstract

Objectives—The aim of this investigation is to determine whether 17 α -hydroxyprogesterone caproate is hydrolyzed, in vitro, to 17 α -hydroxyprogesterone and caproate.

Study design—The in vitro hydrolysis of dual radioactively labeled 17 α -hydroxy-[³H] progesterone [¹⁴C] caproate by human plasma, hepatic and placental S9 fractions as well as recombinant esterases was investigated. The formation of [³H]-17 α -hydroxyprogesterone and [¹⁴C]-caproate were determined using HPLC equipped with an online radioactivity detector. The presence and activity of carboxylesterase and butyrylcholinesterase in the human derived preparations was confirmed by the hydrolysis of their prototypic substrates *p*-nitrophenyl acetate, *p*-nitrophenyl butyrate and butyrylthiocholine, respectively.

Results—The aforementioned human derived preparations hydrolyzed *p*-nitrophenyl acetate, *p*-nitrophenyl butyrate and butyrylthiocholine. However, when 17 α -hydroxyprogesterone caproate was incubated with the human derived preparations under identical experimental conditions neither [³H]-17 α -hydroxyprogesterone nor [¹⁴C]-caproate was detected.

Conclusion—17 α -Hydroxyprogesterone caproate is not hydrolyzed in vitro by the esterase enzymes present in human plasma, liver, preterm or term placenta.

Keywords

Spontaneous preterm labor; 17 α -hydroxyprogesterone caproate; hydrolysis

Introduction

Preterm delivery (<37 weeks of gestation) is a leading cause of perinatal and neonatal morbidity and mortality in the USA and other countries. The prevention of preterm delivery is associated with the use of progestins, either natural (progesterone) or synthetic. The esterification of 17 α -hydroxyprogesterone (17HP) with caproate to form 17 α -hydroxyprogesterone caproate (17HPC) resulted in potentiating and prolonging its weak progestational activity¹. Moreover, the efficacy of 17HPC in treatment of preterm delivery was investigated in a multicenter, randomized controlled trial conducted by the National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units

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(MFMU) Network. The data obtained demonstrated that weekly injections of 17HPC initiated in the second trimester of pregnancy reduced, by 33%, the incidence of preterm delivery in women with history of prior spontaneous preterm birth². However, the mechanism of action of 17HPC remains unclear. As a matter of fact a fundamental question remains unanswered, namely, is 17 HPC a drug or a pro-drug i.e. its pharmacological activity is attributed to one or more of its metabolites.

Earlier investigations of the effect of 17HPC on the endometrium revealed its progestational activity and recommended its administration in two doses³. Subsequently, the administration of 17HPC was compared to that of progesterone in 3 women concluded that the esterification of progesterone prolonged its effect⁴. Moreover, very low concentrations of 17HPC were detected in patients under treatment with the drug for endometrial carcinoma⁵. In addition, traces of 17HP were detected in the urine of pregnant women receiving 1 gram of 17HPC⁶. These earlier reports (Nineteen Fifties) did not offer any explanations of the data.

There are at least two possible pathways for the metabolism of 17HPC: First, hydrolysis of its ester bond by plasma and tissue esterases resulting in the formation of 17HP and caproate; Second, oxidation or conjugation of 17HPC by cytochrome P450 isozymes or other enzymes, respectively. There are two major groups of enzymes that catalyze the hydrolysis of medications in human, namely carboxylesterases (CEs) and butyrylcholinesterase (BChE). CE are widely distributed in human tissues but their activity in the endoplasmic reticulum of the liver is higher⁷. These enzymes hydrolyze a variety of ester-containing drugs and prodrugs such as cocaine and heroin⁷ to their respective acid and alcohol. BChE is one of the major enzymes that hydrolyze natural and synthetic carboxylic or phosphoric acid ester-bond-containing compounds⁸. It is present in the liver, intestinal mucosa, white matter of mammalian central nervous system and in higher levels in plasma⁸. Both CE and BChE are expressed in human placenta^{9,10}.

Therefore, the aim of this investigation was to determine whether 17HPC is hydrolyzed in vitro by human liver, term and preterm placentas, and plasma to 17HP and caproate.

Materials and Methods

Materials

All chemicals and the following recombinant enzymes, equine serum butyrylcholinesterase (eBChE), rabbit (rICE) and porcine liver carboxylesterases (pICE) were purchased from Sigma-Aldrich (St. Louis, Mo). The radioactive isotopes of 17 α -hydroxy [1,2,6,7-³H]-progesterone [1-¹⁴C]caproate, 17 α -hydroxy[1,2,6,7-³H]-progesterone and [1-¹⁴C]caproate were custom synthesized by RTI International (Research Triangle Park, Nc). Pools of human liver S9 preparations were purchased from BD Biosciences (Woburn, Ma).

Methods

Human blood samples were donated by healthy volunteers and plasma preparations were obtained by centrifugation at 500*g* for 10 minutes at 4°C and the supernatant stored at -70 °C until used. Human placentas were obtained from healthy pregnancies at term (n= 8, gestational age, 39-40 weeks) and preterm (n=4, gestational age between 22 and 34 weeks) according to a protocol approved by the Institutional Review Board. Villous tissue was dissected within 5 minutes after delivery, rinsed with ice-cold saline followed by homogenization in 2 volumes of cold 0.1 M potassium phosphate buffer (pH7.4). The homogenate was centrifuged at 10,000*g* for 30 minutes at 4°C, the pellet discarded and the supernatant (S9 fraction) stored -80°C until pooled. S9 fractions from 8 term and 4 preterm placentas were pooled separately and stored at -80°C until used.

Esterase activity

The carboxylesterase activity of human plasma, liver and placental S9 preparations as well as that for recombinant esterases, rICE and pICE, in their hydrolysis of the prototypic substrates *p*-nitrophenyl acetate (*p*NPA), *p*-nitrophenyl butyrate (*p*NPB) was determined. The reaction solution contained one of the following preparations: human plasma, hepatic or placental S9 fractions, recombinant esterases rICE or pICE in 0.1 M potassium phosphate buffer (pH 7.4) containing 2 mM EDTA in a total volume of 1.990 mL. The components were preincubated for 5 min at 37°C and the reaction initiated by the addition of one of the substrates: 10 µL of 1M *p*NPA or *p*NPB. The incubation was continued for 5 minutes and terminated by placing the tubes on ice and the addition of 1 volume of acetonitrile. The formation of the reaction product, *p*-nitrophenol, was determined by a spectrophotometer at a wavelength of 405 nm using a standard curve. The activity of carboxylesterase is expressed as µmol/min per either mL of plasma or mg protein of the human derived tissue preparations or unit of recombinant esterase.

The activity of BChE was determined in a reaction solution containing one of the following: human plasma, hepatic or placental S9 fractions or the recombinant esterase enzyme (eBChE) in 0.1 M potassium phosphate buffer (pH 7.4). The reaction solution was preincubated for 5 min at 37°C followed by the addition of the substrate *S*-butyrylthiocholine iodide (BTC, 10 mM) and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB, 0.5 mM) and terminated after 5 min by placing the tubes on ice and the addition of 1 volume of acetonitrile. The amount of product formed (5-thio-2-nitrobenzoate) was determined spectrophotometrically at 412 nm against a reagent blank containing BTC and DTNB according to the method of Ellman et al.¹¹.

Effects of esterases on 17HPC

The hydrolysis of 17HPC by human plasma, liver and placental S9 and recombinant esterases was determined. The reaction solution (total volume of 1 mL) contained either 1 mg hepatic protein, 2 mg placental protein, 0.5ml plasma or 0.5 unit of recombinant esterase in 0.1 M potassium phosphate buffer (pH 7.4). The solution was preincubated for 5 min at 37°C and the reaction initiated by the addition of 17HPC at a final concentration of 50 µM (³H: 0.6 µCi/µmol, ¹⁴C: 0.2 µCi/µmol) and shaken for 60 min at the same temperature. Reactions with 0 min incubation served as controls. All reactions were conducted in triplicates. The reaction was terminated by placing the tubes on ice and the addition of 100 µL ice-cold 10% trichloroacetic acid (TCA). Aliquots of the reaction solution (100 µL) were added to the scintillation cocktail (Research Products International, Mount Prospect, IL) and the radioactivity determined in a liquid scintillation analyzer (Packard Instruments, Meriden, Ct). The remaining reaction solution was extracted twice with methylene chloride (MtCl). The MtCl layers were combined, dried under a stream of nitrogen and reconstituted in 150 µL of acetonitrile. Recovery of total radioactivity was > 95%. The substrate (17HPC) and products of the reactions (17HP and caproate) were separated by HPLC, identified by their retention times and the corresponding radionuclide (³H for 17HP and ¹⁴C for caproate). The retention times of the radioisotopes of the three compounds (standards) were as follows: 17HPC (32.9 min), 17HP (21.9 min) and caproate (9.0 min).

HPLC

The HPLC system consisted of a Waters 600E multisolvent delivery system, a 2487 dual λ absorbance detector and a 717 autosampler controlled by Millennium chromatography manager (Waters, Milford, Ma). The column was a Waters Symmetry C18 column (5 µm, 150 × 4.6 mm). The mobile phase consisted of: A; 0.25% acetic acid/acetonitrile, 70/30 by volume and B; H₂O/acetonitrile, 10/90 by volume. Samples were eluted using a gradient initially 100% A for 5 min followed by a linear increase to 100% B over a period of 30 min

then maintained for an additional 10 min. The flow rate was 1 mL/min. Radioactivity was determined by a β -RAM flow-through on-line detector (model 4, IN/US Systems, Tampa, FL) connected to the HPLC system and controlled by a ScintFlow SA software program. The detection limits of [^{14}C]caproate, [^3H]17HP and [^3H , ^{14}C]17HPC were determined by adding known amounts of the above compounds to human tissue preparations in concentrations ranging between 1 and 2000 femto moles. The samples were extracted and analyzed as described above.

Results

Figure 1 illustrates the position of radioactive nuclides (tritium and ^{14}C) in the [^3H , ^{14}C]-17HPC, [^3H]-17HP and [^{14}C]caproate.

The HPLC method developed for this investigation revealed clear separation of 17HPC from 17HP and caproate (Fig. 2A & 2B). The detection limits of all the compounds were in the femto moles range between 300 and 1200.

The activities of the two recombinant carboxylesterases in hydrolyzing *p*NPA and *p*NPB as well as that for the recombinant butyrylcholinesterase in hydrolyzing BTC were determined. The data obtained (Table 1) revealed that the activity of recombinant porcine hepatic carboxylesterase in hydrolyzing *p*NPA was approximately 50 times that for the rabbit liver enzyme. However, for the same enzyme, hydrolysis of *p*NPB by the rabbit liver was 5 times that of porcine liver. These data indicate that the activity of the recombinant enzymes is different and is species dependent. Moreover, equine butyrylcholinesterase hydrolyzed its substrate BTC but at a lower rate than that observed for carboxylesterases.

The above data demonstrates that the reaction conditions used in this investigation is suitable for determining the activity of these enzymes in hydrolyzing compounds containing an ester bond.

The activity of human derived preparations in the hydrolysis of the prototypic substrates of carboxylesterases and butyrylcholinesterase were investigated under the same experimental conditions utilized for the recombinant enzymes. The data obtained (Table 2) revealed that all the human derived preparations hydrolyzed their prototypic substrates. Moreover, the activities of human hepatic carboxylesterases were 50-70 times that of human preterm or term placentas. However, the activity of these derived preparations in hydrolysis of BTC did not reflect a significant difference between organs.

It is difficult to compare the activity of human plasma preparations with those derived from tissues because the denominator used is per "ml" for the former and per "mg protein" for the latter. Nevertheless, the activity of carboxylesterases in human plasma was 30 times that of butyrylcholinesterase.

The activity of all the above mentioned human derived preparations was investigated for their hydrolysis of [^3H , ^{14}C]-17HPC under the same experimental conditions used for the prototypic substrates. Neither [^3H]-17HP nor [^{14}C]-caproate was detected (femto mole levels) in presence of human plasma, liver or placental S9 fractions (Fig. 2C & 2D). Moreover, all recombinant esterases (0.5 unit/mL) did not hydrolyze [^3H , ^{14}C]-17HPC.

Taken together, data presented in this report indicate that 17-HPC was not hydrolyzed in vitro under the experimental conditions used.

Comment

The goal of an ongoing project in our laboratory is to better understand the role of 17HPC in treatment of preterm delivery. To date, it is unclear whether 17HPC is a drug or a prodrug. If a prodrug, then there are at least two possible metabolic pathways leading to its conversion to a drug: First, hydrolysis of the ester bond by plasma and tissue esterases to 17HP and caproate; Second, its derivatization either by its oxidation (cytochrome P450 isozymes) or by its conjugation (e.g. with glucuronic acid). The aim of this investigation was to determine whether 17HPC is hydrolyzed, in vitro, to 17HP and caproate. To achieve this goal, a dual radioactive labeled 17HPC, tritium in the progesterone nucleus and ^{14}C in the caproate acyl chain, was utilized to enhance the detection limits of the products formed to the level of femto moles.

The hydrolysis of 17HPC by carboxylesterases and butyrylcholinesterases which are widely distributed in human plasma and tissues was investigated. Moreover, the hydrolysis of 17HPC by recombinant preparations of these esterases was also investigated to compare their activities to those of human derived preparations. To ensure that the experimental conditions used are optimal for hydrolysis of ester bonds by these enzymes, the hydrolysis of their prototypic substrates *p*NPA, *p*NPB and BTC was determined. It should be noted here that the lack of commercially available recombinant human esterases compelled us to use those of laboratory animals which have DNA homology to human.

Data on the rate of *p*NPA hydrolysis by recombinant liver carboxylesterases revealed that the porcine preparation was approximately 50 times higher than that of rabbit. However, the activity of recombinant rabbit liver carboxylesterase in hydrolyzing *p*NPB was 5 times that of porcine liver. On the other hand, the activity of recombinant equine butyrylcholinesterase in the hydrolysis of BTC was much lower than that of carboxylesterases (Table 1). The differences observed in the rate of hydrolysis of the prototypic substrates by the recombinant esterases are most likely due to inherent properties of the species.

The rates of hydrolysis of the prototypic substrates *p*NPA and *p*NPB by human derived preparations varied between organs. The carboxylesterase activity of human liver was approximately 50 times that of placenta. In general, the activities of carboxylesterases in the various human derived preparations were in the following descending order: human liver>plasma>placenta (Table 2). Similar data on the higher activity of hepatic CYP450 isozymes than that of placenta were reported¹². Moreover, the activity of human plasma in the hydrolysis of BTC was higher than tissue (liver and placentas) preparations. Taken together, it is apparent that human plasma and tissue (liver and placenta) derived preparations exhibit hydrolysis of the prototypic substrates of carboxylesterases and butyrylcholinesterases under the experimental conditions used.

An HPLC method was developed to separate the substrate 17HPC from its hydrolysis products 17HP and caproate. The products formed were identified by their retention times and their radioactive nuclide namely, ^3H in 17HP and ^{14}C in the caproate (Fig 2A & B). Data obtained in this investigation revealed that human plasma and tissue preparations did not hydrolyze 17HPC as revealed by the lack of 17HP or caproate formation (Fig. 2C&D). Data cited here on the lack of 17HPC hydrolysis by term placental S9 fraction is in agreement with that utilizing term placental homogenates¹³. Moreover, in this investigation, it was demonstrated that 17HPC is not hydrolyzed by a number of the recombinant esterases.

Taken together, it can be concluded that 17HPC is not hydrolyzed in vitro by the following: several recombinant esterases, human plasma, placental and hepatic derived preparations. Therefore, if the lack of 17HPC hydrolysis is also true in vivo, it can be concluded that

17HPC is a drug and not a prodrug. However, traces of 17HP were detected in urine of pregnant women under treatment with 17HPC⁶. It is unclear whether the reported “traces” of 17HP are a result of its de novo synthesis by maternal ovary or fetal adrenal. Alternatively, in the absence of a quantitative determination of the “traces” of 17HP, its presence as an “impurity” in the 17HPC preparation used at that time (1955) can not be ruled out.

The absence of 17HPC hydrolysis in vitro does not rule out its possible oxidation in vivo or in vitro by cytochrome P450 isozyme(s) or its conjugation with, for example, glucuronic acid.

In conclusion, data obtained from this investigation demonstrated that 17HPC is not hydrolyzed in vitro by either human plasma or tissue derived preparations from liver and placenta. However, it does not rule out either its hydrolysis in vivo or its metabolism by a metabolic pathway that does not begin with its hydrolysis.

Acknowledgments

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References

1. Reifstein EC. Introduction of marked as well as prolonged biologic activity by esterification-17-Alpha-Hydroxyprogesterone caproate, a unique progestational compound. *Fertility & Sterility*. 1957; 8:50–78. [PubMed: 13405048]
2. Meis PJ, Klebanoff M, Thom E, Dombrowski MP, Sibai B, Moawad AH, et al. Prevention of recurrent preterm delivery by 17-alpha-hydroxyprogesterone caproate. *N Engl J Med*. 2003; 348:2379–85. [PubMed: 12802023]
3. Boschann HW. Effect of 17-alpha-hydroxyprogesterone caproate on human endometrium. *Arztl Wochensh*. 1954; 9:591–3. [PubMed: 13171236]
4. Davis ME, Wied GL. 17-Alpha-hydroxyprogesterone-caproate: a new substance with prolonged progestational activity; a comparison with chemically pure progesterone. *J Clin Endocrinol Metab*. 1955; 15:923–30. [PubMed: 13242645]
5. Onsrud M, Paus E, Haug E, Kjorstad K. Intramuscular administration of hydroxyprogesterone caproate patients with endometrial carcinoma. *Acta Obstet Gynecol Scand*. 1985; 64:519–523. [PubMed: 2932883]
6. Langecker H. The fate of 17alpha-hydroxyprogesterone and its 17-caproate in the human organism. *Arch Exper Path and Pharmacol*. 1955; 225:309.
7. Brzezinski MR, Spink BJ, Dean RA, Berkman CE, Cashman JR, Bosron WF. Human liver carboxylesterase hCE-1: Binding specificity for cocaine, heroin and their metabolites and analogs. *Drug Metab Dispos*. 1997; 25:1089–96. [PubMed: 9311626]
8. Massoulié J, Pezzementi L, Bon S, Krejci E, Vallette FM. Molecular and cellular biology of cholinesterases. *Prog Neurobiol*. 1993; 41:31–91. [PubMed: 8321908]
9. Yan B, Matoney L, Yang D. Human carboxylesterases in term placenta: enzymatic characterization, molecular cloning and evidence for the existence of multiple forms. *Placenta*. 1999; 20:599–607. [PubMed: 10452915]
10. Sastry BVR. Human placental cholinergic system. *Biochem Pharmacol*. 1997; 53:1577–86. [PubMed: 9264309]
11. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 1961; 7:88–95. [PubMed: 13726518]
12. Pasanen M, Pelkonen O. Xenobiotic and steroid-metabolizing monooxygenases catalysed by cytochrome P450 and glutathione S-transferase conjugations in the human placenta and their relationships to maternal cigarette smoking. *Placenta*. 1990; 11:75–85. [PubMed: 2326240]

13. Wiener M, Lupu CI, Plotz EJ. Metabolism of 17 α -hydroxyprogesterone-4-¹⁴C-17 α -caproate by homogenates of rat liver and human placenta. *Acta Endocrinologica*. 1961; 36:511–526. [PubMed: 13785108]

Figure 1.

The position of radioactive nucleotides in the utilized [^3H , ^{14}C]-17HPC and its hydrolysis products [^3H]-17HP and [^{14}C]-caproate. T indicates [^3H] and “*” indicates [^{14}C].

Figure 2.
Representative HPLC chromatograms for:

- A.** Standards of $[^{14}\text{C}]$ -caproate and $[^3\text{H}, ^{14}\text{C}]$ -17HPC monitored in the ^{14}C channel.
- B.** Standards of $[^3\text{H}]$ -17HP and $[^3\text{H}, ^{14}\text{C}]$ -17HPC monitored in the tritium channel.
- C.** Reaction solution of $[^3\text{H}, ^{14}\text{C}]$ -17HPC after incubation in presence of human liver S9 preparation monitored in ^{14}C channel.
- D.** Same as C but monitored in the tritium channel.

Table 1

The activities of recombinant carboxylesterase and butyrylcholinesterase.

Enzyme	Substrate	Activity ($\mu\text{mol}/\text{min}\cdot\text{unit of esterase}$)		
		rabbit liver carboxylesterase	porcine liver carboxylesterase	equine serum butyrylcholinesterase
Carboxylesterase	<i>p</i> NPA	127.9 $\pm$ 7.0	5974.8 $\pm$ 565.7	-
	<i>p</i> NPB	205.2 $\pm$ 10.9	40.6 $\pm$ 5.0	-
Butyrylcholinesterase	BTC	-	-	0.88 $\pm$ 0.03

Each value is the means of triplicates $\pm$ SD.

-: not applicable

Table 2

The activity of carboxylesterase and butyrylcholinesterase in human plasma and S9 fractions of liver and placenta

Enzyme	Substrate	human liver S9*	human placental S9*		human plasma [#]
			preterm	term	
Carboxylesterase	pNPA	677.5±5.4	15.9±0.6	22.6±0.8	273.0±19.3
	pNPB	708.4±48.6	10.2±0.3	6.3±0.6	253.5±9.2
Butyrylcholinesterase	BTC	0.03±0.001	0.03±0.001	0.02±0.0003	8.0±0.08

Each value is the means of triplicates ± SD.

* μmol/min per mg protein;

[#] μmol/min per milliliter plasma