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The (+)- and (−)-gossypols potently inhibit human and rat 11β-hydroxysteroid dehydrogenase type 2☆

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ABSTRACT

Gossypol has been proven to be a very effective male contraceptive. However, clinical trials showed that the major side effect of gossypol was hypokalemia. Gossypol occurs naturally as enantiomeric mixtures of (+)-gossypol and (−)-gossypol. The (−)-gossypol is found to be the active component of antifertility. 11β-Hydroxysteroid dehydrogenase 2 (11βHSD2) has been demonstrated to be a mineralocorticoid receptor (MR) protector by inactivating active glucocorticoids including corticosterone (CORT) in rats, and therefore mutation or suppression of 11βHSD2 causes hypokalemia and hypertension. In the present study, the potency of gossypol enantiomers was tested for the inhibition of 11βHSD1 and 2 in rat and human. Both (+) and (−)-gossypols showed a potent inhibition of 11βHSD2 with the half maximal inhibitory concentration (IC_{50}) of 0.61 and 1.33 μM for (+) and (−)-gossypols, respectively in rats and 1.05 and 1.90 μM for (+) and (−)-gossypols, respectively in human. The potency of gossypol to inhibit 11βHSD1 was far less; the IC_{50} was ≥100 μM for racemic gossypol. The gossypol-induced hypokalemia is likely associated with its potent inhibition of kidney 11βHSD2.

1. Introduction

Glucocorticoids have a wide range of physiological and pharmacological roles in mammalian functions [1,2]. Intracellular levels of glucocorticoids (cortisol in humans or corticosterone, CORT, in rats) are regulated by 11β–hydroxysteroid dehydrogenase (11βHSD), which has two known isoforms. Type I 11βHSD (11βHSD1) is an $NADP^+$/NADPH dependent oxidoreductase, catalyzing the interconversion of 11β-hydroxyl steroids and 11-keto steroids (such as 11-dehydrocorticosterone, 11DHC, in rats) and is most abundantly expressed in glucocorticoid target tissues such as liver, fat and testis [3]. 11βHSD1 is a low-affinity high capacity enzyme with a Km of 2 μM [4]. 11βHSD1 behaves primarily as a reductase in liver and fat, thus regenerating active glucocorticoids from circulatory inactive 11-keto glucocorticoids [3]. Increased 11βHSD1 activity in fat tissues has been hypothesized to be the main cause of metabolic syndrome [3] and has motivated many scientists and companies to develop 11βHSD1 reductase inhibitors to treat metabolic syndrome and its related diseases [3]. The other isoform is type II 11βHSD (11βHSD2), which is an NAD^+ dependent unidirectional oxidase and inactivates glucocorticoids thus protecting mineralocorticoid receptor from occupance by natural glucocorticoids [5]. Mutation of 11βHSD2 gene in human causes a syndrome named apparent mineralocorticoid excess (AME) featuring hypertension and hypokalemia in which circulatory aldosterone levels are subnormal [6]. Inhibition of 11βHSD2 by chemicals such as glycyrrhentic acid, a component of licorice can cause a clinical condition similar to AME [7].

Gossypol, a lipid soluble polyphenolic compound purified from cotton seeds, has been shown to be very effective as male contraceptive [8]. However, the use of gossypol as a male contraceptive is limited in the clinical setting because of hypokalemia, one of the severe side effects observed in some Chinese men who participated in the clinical trial [8]. Gossypol naturally occurs as enantiomeric mixtures with (+)-gossypol and (−)-gossypol. It has been found that only (−)-gossypol exerts the antifertility action [9,10]. Thus (+)-gossypol has been thought to be the unwanted component.

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The mechanism underlying gossypol-induced hypokalemia has been hypothesized to be related to its effects on renal 11βHSD [11]. Racemic gossypol acetic acid was found to be a competitive inhibitor with a Ki of 67 ± 5 μM for $NADP^+$-dependent 11βHSD1 in guinea pig kidney microsomes, the enzyme that has not been found to be involved in renal potassium secretion subsequently. Enantiomer (+)-gossypol seemed a little more potent than (−) enantiomer in the inhibition of 11βHSD1 [11]. The inhibition of racemic gossypol on 11βHSD1 was also found on human 11βHSD1 oxidase with IC_{50} of 147 μM [12]. However, the inhibition of 11βHSD1 has been found to be not associated with hypokalemia. In fact, the inhibition of 11βHSD1 may be beneficial in treating metabolic syndrome or type 2 diabetes. After the 11βHSD2 isoform was cloned, its mutation was found to be associated with AME, a syndrome featuring hypokalemia and hypotension [13]. Racemic gossypol was also shown to inhibit NAD^+ dependent 11βHSD2 in guinea pig with a Ki of 34 μM [14]. However, it is unclear for the potency of gossypol enantiomers to inhibit 11βHSD1 and 2 isoforms in human and other species. The object of the present study was to compare the potency of gossypol enantiomers in the inhibition of 11βHSD1 and 2 isoforms from rat and human species. All animal procedures were approved by the Rockefeller University's Animal Care and Use Committee (protocol# 07080).

2. Materials and methods

2.1. Chemicals and animals

[1,2-^{3}H]Corticosterone (^{3}H-CORT), specific activity, 40 Ci/mmol) was purchased from Dupont-New England Nuclear (Boston, MA). [1,2-^{3}H]11-dehydrocorticosterone (^{3}H-11DHC) was prepared from labeled ^{3}H-CORT as described earlier [15]. Cold CORT and 11DHC were purchased from Steraloids (Wilton, NH). Racemic, (−)- and (+)-gossypols were the gifts from Dr. Samuel S. Koide in Population Council. Male Sprague–Dawley rats (250–300 g) were purchased from Charles River Laboratories (Wilmington, MA). Human liver microsomes were purchased from Gentest (Woburn, MA).

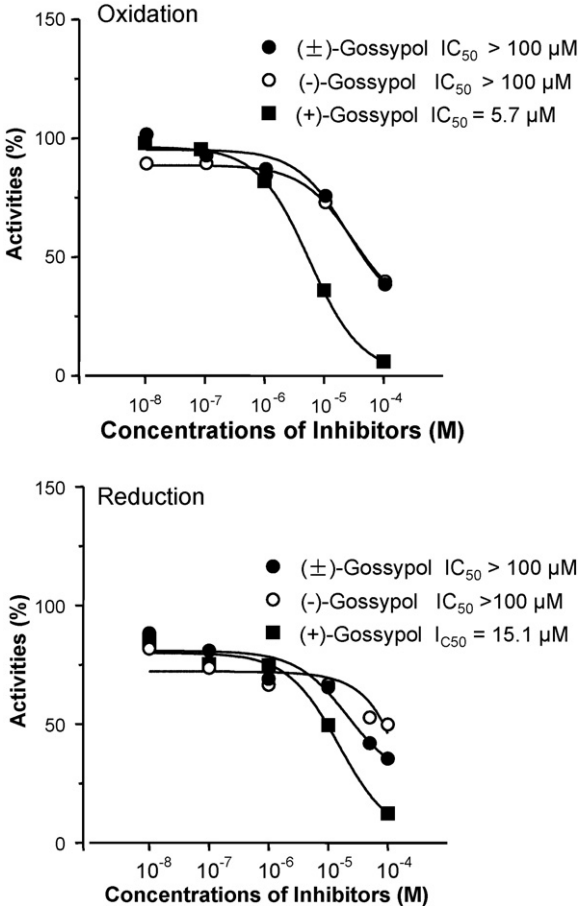

Fig. 1. Inhibition of gossypol enantiomers on rat liver microsome 11βHSD1 oxidase and reductase activities. Two μg (for oxidase) or 5 μg (for reductase) of rat liver microsomes were incubated with different concentrations of gossypols and 25 nM substrates in the presence of 0.2 mM $NADP^+$ for oxidase and 0.2 mM NADPH plus 0.2 mM G6P for reductase activities for 30 min. Values from 6 samples in a duplicate assay were represented.

Table 1
IC50s for gossypol enantiomers and carbenoxolone on 11βHSD1 and 11βHSD2 (n = 4).

Chemicals	Rat liver microsomes 11βHSD1		Rat kidney microsomes 11βHSD2 (μM)
	Oxidase (μM)	Reductase (μM)	
(±)-Gossypol	>100	>100	0.88
(−)-Gossypol	>100	>100	1.33
(+)-Gossypol	5.7 ± 1.2	15.1	0.61
Carbenoxolone	0.04	0.65	0.05

Chemicals	Rat intact Leydig cells 11βHSD1	
	Oxidase (μM)	Reductase (μM)
(±)-Gossypol	NA	NA
(−)-Gossypol	>100	>100
(+)-Gossypol	>100	>100
Carbenoxolone	19.2	>100

Chemicals	Human liver microsomes 11βHSD1		Human kidney microsomes 11βHSD2 (μM)
	Oxidase (μM)	Reductase (μM)	
(±)-Gossypol	>100	>100	2.14
(−)-Gossypol	>100	>100	1.90
(+)-Gossypol	>100	80.6	1.05
Carbenoxolone	0.13	1.81	0.02

The data were repeated by four times.

2.2. Cell isolation

Rats were sacrificed by asphyxiation with CO_2. Testes were removed and Leydig cells were purified as described previously [16]. Purities of Leydig cell fractions were evaluated by histochemical staining for 3β-hydroxysteroid dehydrogenase (3βHSD) activity, with 0.4 mM etiocholanolone as the steroid substrate [17]. More than 95% Leydig cells were intensely stained.

2.3. Preparation of microsomal protein

Microsomal preparations of rat liver and kidney were prepared as described previously [18]. Pellets were resuspended and protein contents were measured. Microsomes were used for measurement of 11βHSD activities.

2.4. 11βHSD assay

11βHSD activity assay tubes contained 25 nM (within the range of physiological levels of CORT). [^{3}H]-CORT or [^{3}H]-11DHC were used as substrates to measure either 11βHSD1 oxidase or reductase activity. Intact Leydig cells were preincubated with gossypols for 2 min and then [^{3}H]-CORT or [^{3}H]-11DHC were added into the cells for measuring either 11βHSD1 oxidase or reductase activity. For microsomal preparation, the liver and kidney microsomes were incubated with substrates, 0.2 mM $NADP^+$ (for 11βHSD1 oxidase activity) or 0.2 mM NADPH plus 0.2 mM glucose-6-phosphate (for 11βHSD1 reductase activity) or 0.2 mM NAD^+ (for 11βHSD2 activity). The reactions were stopped by adding 2 ml ice-cold ether. The steroids were extracted, and the organic layer was dried under nitrogen. The steroids were separated chromatographically on thin layer plates in chloroform and methanol (90:10, v/v), and the radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC). The percentage conversion of CORT to 11DHC and 11DHC to CORT was calculated by dividing the radioactive counts identified as 11DHC (or CORT, respectively) by the total counts associated with CORT plus 11DHC.

2.5. Statistics

Each experiment was repeated twice to three times. Data were subjected to nonlinear analysis by GraphPad (Version 3, GraphPad Software Inc., San Diego, CA), and 50% maximal inhibitory concentrations (IC_{50}) was calculated. Data were subjected to analysis by one-way ANOVA followed by DUNCAN multiple comparisons testing to identify significant differences between groups when three and more groups were calculated. All data are expressed as means ± SEM. Differences were regarded as significant at $P < 0.05$.

3. Results

3.1. Effects of gossypol enantiomers on 11βHSD1 activities in rat liver microsomes and intact Leydig cells

11βHSD1 is an oxidoreductase, therefore both oxidase and reductase activities were measured in the presence of cofactors ($NADP^+$ for oxidase) and (NADPH plus G6P for reductase). As shown in Table 1, gossypol enantiomers were weak 11βHSD1 oxidase and reductase inhibitors when compared to carbenoxolone, which is a potent inhibitor of 11βHSD1 oxidase (IC_{50} = 0.041 μM) and reductase (IC_{50} = 0.65 μM) (Table 1). Gossypol enantiomers have different inhibitory potencies. (+)-Gossypol was a more potent inhibitor than

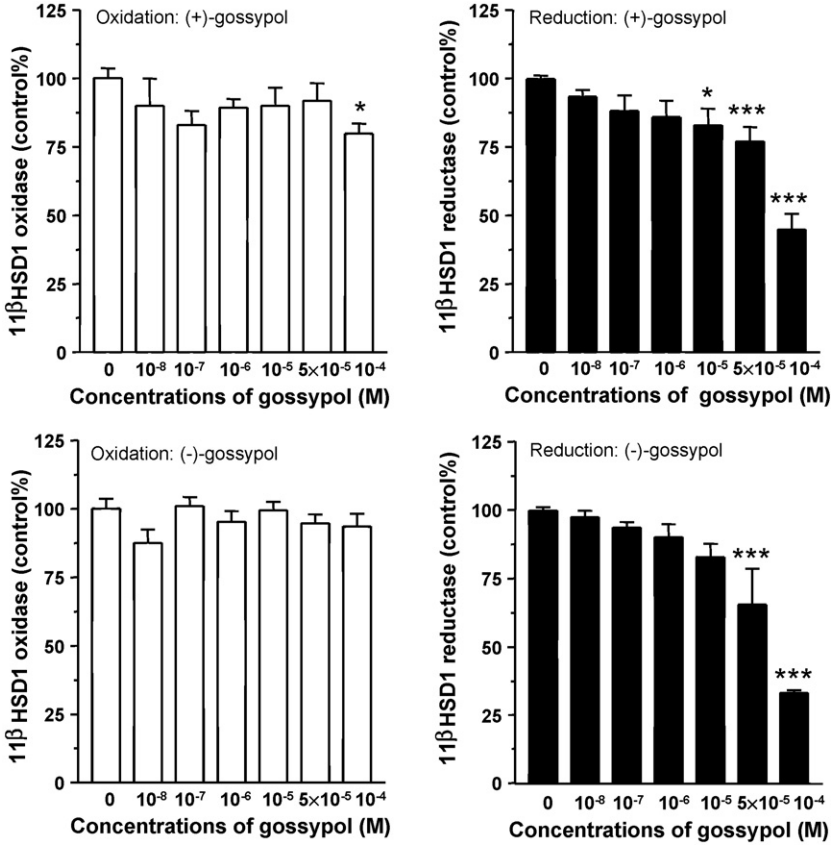

Fig. 2. Inhibition of gossypol enantiomers on rat intact Leydig cell 11βHSD1 oxidase and reductase activities. The cells of 0.05×10^6 (30 min for oxidase) and 0.05×10^6 (2 h for reductase) were incubated with substrates using endogenous cofactors. Values from 4 samples in a duplicate assay were represented. Mean ± SEM, $n = 4$. *,**,***Significant difference compared to zero concentration group at $P < 0.05$, 0.01, 0.001, respectively.

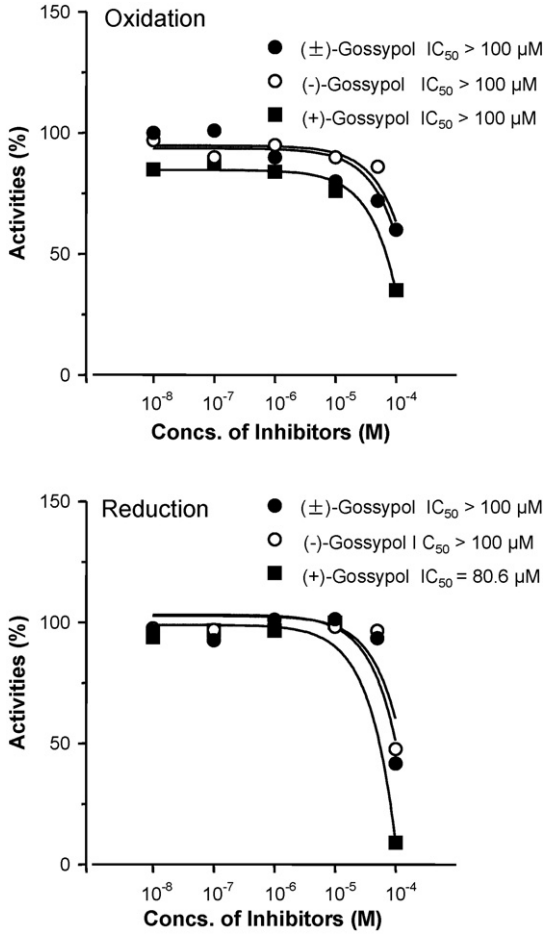

Fig. 3. Inhibition of gossypol enantiomers on human liver microsome 11βHSD1 oxidase and reductase activities. One microgram of human liver microsomes were incubated with different concentrations of gossypols and 25 nM substrates in the presence of 0.2 mM $NADP^+$ for oxidase for 1 h and 2 μg of microsome proteins with 0.2 mM NADPH plus 0.2 mM G6P for reductase activities for 2 h. Values from 6 samples in a duplicate assay were represented.

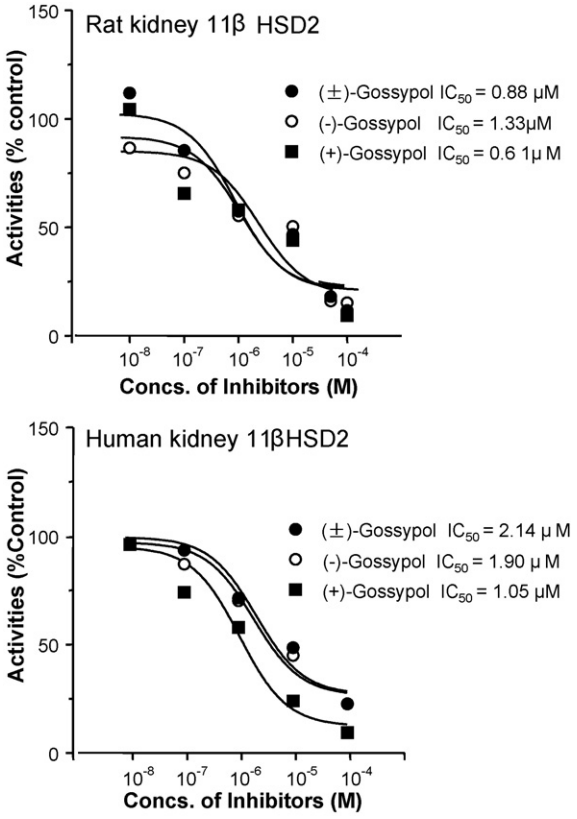

Fig. 4. Inhibition of gossypol enantiomers on rat and human kidney microsome 11βHSD2 oxidase and reductase activities. Five μg of rat kidney or 40 μg of human kidney microsomes were incubated with different concentrations of gossypols and 25 nM substrates in the presence of 0.2 mM NAD^+ for oxidase for 1–3 h. Values from 4 samples in a duplicate assay were represented.

(−)-gossypol and racemic gossypol in the inhibition of 11βHSD1 oxidase activity (with IC_{50} = 5.7 μM) and reductase activity (with IC_{50} = 15.1 μM) in rat liver microsomes (Fig. 1). The inhibition of 11βHSD1 oxidase and reductase activities were also measured in intact rat Leydig cells (Table 1 and Fig. 2). Gossypol enantiomers were weaker inhibitors for 11βHSD1 oxidase than for reductase (Fig. 2). Compared to (−)-gossypol, (+)-gossypol was a more potent inhibitor for 11βHSD1 activities in intact Leydig cells. These data indicate that gossypol enantiomers are weak 11βHSD1 inhibitors.

3.2. *Effects of gossypol enantiomers on 11βHSD1 activities in human liver microsomes*

The effects of gossypol enantiomers on human liver microsomal 11βHSD1 oxidase and reductase activities were also measured. As expected, carbenoxolone was a potent inhibitor for human 11βHSD1 (Table 1). In contrast to rat liver 11βHSD1 oxidase and reductase activities, human liver 11βHSD1 from liver microsomes were more resistant to the inhibition by gossypol enantiomers, with IC_{50} around 100 μM or over (Fig. 3).

3.3. *Effects of gossypol enantiomers on 11βHSD2 activities in rat and human kidney microsomes*

The effects of gossypol enantiomers on rat and human kidney microsomal 11βHSD2 activities were measured. Gossypol enantiomers showed potent inhibition of both rat and human 11βHSD2 (Fig. 4) with (+)-gossypol slightly more potent to both human and rat 11βHSD2. The finding suggests that both gossypol enantiomers are potent 11βHSD2 inhibitor.

4. Discussion

The present study showed that gossypol enantiomers inhibited 11βHSD2 in rat and human with IC_{50} around 1 μM. In contrast, they inhibited 11βHSD1 with IC_{50} around 15 μM or greater. This indicates that gossypol enantiomers are more specific inhibitors for 11βHSD2 and could be the cause of gossypol-induced hypokalemia.

Gossypol enantiomers including (+)-gossypol, (−)-gossypol and the racemic gossypol were competitive inhibitors of 11βHSD1 oxidase and reductase. Song et al. previously reported that the IC_{50} for the inhibition of human liver 11βHSD1 oxidase activity by racemic gossypol was 147 μM [12]. The present study confirmed this finding and further showed that each enantiomer had similar effects (Fig. 3). We found that (+)-gossypol enantiomer inhibited rat 11βHSD1 (IC_{50} = 5.7 μM) more potently than human (with $IC_{50} \geq 100$ μM) in the present study. Others reported that the IC_{50} of inhibition to guinea pig 11βHSD1oxidase by racemic gossypol was 1.114 μM [14]. Therefore, (+)-gossypol is more potent in inhibiting 11βHSD1 oxidase than (−)- or racemic gossypol.

Previously, the inhibition of 11βHSD1 oxidase by gossypol was evaluated in guinea pig kidney [11,12,14]. In contrast, 11βHSD1 in the liver behaves as a primary reductase in intact cells or *in vivo* [18]. The potency of gossypol enantiomers to inhibit 11βHSD1 reductase has never been evaluated in the past because 11βHSD1 reductase activity was readily lost [11,12,14]. Recently, it has

been reported that 11βHSD1 behaves a reductase due to the presence of hexose-6-phosphate dehydrogenase (H6PDH), which is co-localized in the smooth endoplasmic reticulum (SER) luminal space [19–21]. H6PDH is a component of a SER pentose phosphate pathway in many cells, converting glucose-6-phosphate to 6-phosphogluconate and generating NADPH [19–21]. The generated NADPH feeds 11βHSD1 to be a reductase. The evidence that H6PD is a key regulator of 11β-HSD1 in liver and fat tissue has been obtained from study on the H6PD knockout mouse, in which the lack of H6PDH resulted in a switch in 11βHSD1 activity from reductase to dehydrogenase in mouse liver [22]. The addition of G6P to microsome can restore 11βHSD1 reductase, thus the inhibition of 11βHSD1 can be determined. We found that the potency of inhibition to 11βHSD1 reductase by gossypol enantiomers was similar to that of inhibition to 11βHSD1 oxidase, with rat 11βHSD1 reductase more sensitive to the inhibition than human one by (+)-gossypol. However, when measuring the 11βHSD1 activity in intact Leydig cells, (+) and (−)-gossypols were inactive in inhibiting 11βHSD1 oxidase.

Apparently, gossypol enantiomers potently inhibit rat and human 11βHSD2 with IC_{50} of 0.6–2 μM. The IC_{50}s of gossypol enantiomers inhibiting human 11βHSD2 are within the blood levels of gossypol in clinical use as male contraceptive. A recent study using low doses (7.5 mg/day) of (±)gossypol showed that blood gossypol levels reached 186 ± 113 ng/mL (about 0.36 ± 0.22 μM) after 12 weeks of treatment [23]. However, the large-scale clinical trials performed in 1980 used much higher gossypol doses (20 mg/day) which may lead much higher blood gossypol levels [8]. Similarly to the inhibition of 11βHSD1, (+)-gossypol seemed a little more potent than (−)-enantiomer in the inhibition of 11βHSD2. The mechanism underlying gossypol-induced hypokalemia has been hypothesized to be caused by its inhibition of renal 11βHSD [11] although at that time they studied the inhibition of 11βHSD1. After the 11βHSD2 isoform was cloned, racemic gossypol was also found to inhibit NAD^+ dependent 11βHSD2. Racemic gossypol inhibited guinea pig kidney 11βHSD2 with IC_{50} of 50.2 μM [14]. However, the present study showed that rat and human 11βHSD2s were sensitive to the gossypol inhibitors with IC_{50} of 0.6–2 μM. This potent inhibition of 11βHSD2 in kidney could explain why it induces hypokalemia in humans. One of consequence of inhibition of 11βHSD2 is hypertension. However, clinical trials did not found that gossypol treatment caused hypertension [8]. However, because gossypol also directly dilates the blood vessels, the effects by the inhibition of 11βHSD2 could be offset its direct action [24].

A more recent clinical trial with reduced dose of gossypol regime proved that this reduced regime of gossypol remained to be very effective as a male contraceptive without side effects of hypokalemia [23]. To further reduce this effect, one of selections is to choose (−)-gossypol since (−)-gossypol is the only active component to inhibit spermatogenesis in racemic gossypol while (+)-gossypol is inactive [9,10]. It is apparent that the mechanism of gossypol-induced suppression of spermatogenesis and the inhibition of 11βHSD2 is different since the (+)-gossypol is about two-fold potent in inhibiting 11βHSD2 but has no inhibitory effect on fertility [9,10]. Therefore, use of (−)-gossypol can reduce the side effects via inhibiting 11βHSD2.

In conclusion, the present study demonstrated (+) and (−)-gossypols are potent inhibitors of rat and human 11βHSD2. They inhibit 11βHSD1 at much higher concentrations.

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References

[1] S.A. Berger, T.J. Cole, W. Schmid, G. Schutz, Molecular genetic analysis of glucocorticoid and mineralocorticoid signaling in development and physiological processes, Steroids 61 (4) (1996) 236–239.
[2] P. Gass, H.M. Reichardt, T. Strekalova, F. Henn, F. Tronche, Mice with targeted mutations of glucocorticoid and mineralocorticoid receptors: models for depression and anxiety? Physiol. Behav. 73 (5) (2001) 811–825.
[3] J.W. Tomlinson, E.A. Walker, I.J. Bujalska, N. Draper, G.G. Lavery, M.S. Cooper, M. Hewison, P.M. Stewart, 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response, Endocr. Rev. 25 (5) (2004) 831–866.
[4] V. Lakshmi, C. Monder, Purification and characterization of the corticosteroid 11β-dehydrogenase component of the rat liver 11β-hydroxysteroid dehydrogenase complex, Endocrinology 123 (5) (1988) 2390–2398.
[5] P.C. White, T. Mune, A.K. Agarwal, 11β-Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess, Endocr. Rev. 18 (1) (1997) 135–156.
[6] P.C. White, T. Mune, F.M. Rogerson, K.M. Kayes, A.K. Agarwal, Molecular analysis of 11 beta-hydroxysteroid dehydrogenase and its role in the syndrome of apparent mineralocorticoid excess, Steroids 62 (1) (1997) 83–88 (Review) (43 refs.).
[7] C.R. Edwards, P.M. Stewart, D. Burt, L. Brett, M.A. McIntyre, W.S. Sutanto, E.R. deKloet, C. Monder, Localisation of 11β-hydroxysteroid dehydrogenase—tissue specific protector of the mineralocorticoid receptor, Lancet 2 (8618) (1988) 986–989.
[8] S.Z. Qian, Z.G. Wang, A potential antifertility agent for males, Ann. Rev. Pharmacol. Toxicol. 24 (1984) 329–360.
[9] P.J. Den Boer, J.A. Grootegoed, Differential effects of (+)- and (−)-gossypol enantiomers on LDH-C4 activity of hamster spermatogenic epithelium in vitro, J. Reprod. Fertil. 83 (2) (1988) 701–709.
[10] S.A. Matlin, R. Zhou, G. Bialy, R.P. Blye, R.H. Naqvi, M.C. Lindberg, (−)-Gossypol: an active male antifertility agent, Contraception 31 (2) (1985) 141–149.
[11] G.W. Sang, B. Lorenzo, M.M. Reidenberg, Inhibitory effects of gossypol on corticosteroid 11-beta-hydroxysteroid dehydrogenase from guinea pig kidney: a possible mechanism for causing hypokalemia, J. Steroid Biochem. Mol. Biol. 39 (2) (1991) 169–176.
[12] D. Song, B. Lorenzo, M.M. Reidenberg, Inhibition of 11 beta-hydroxysteroid dehydrogenase by gossypol and bioflavonoids, J. Lab. Clin. Med. 120 (5) (1992) 792–797.
[13] P.C. White, T. Mune, F.M. Rogerson, K.M. Kayes, A.K. Agarwal, 11β-Hydroxysteroid dehydrogenase and its role in the syndrome of apparent mineralocorticoid excess, Pediatr. Res. 41 (1) (1997) 25–29.
[14] M.S. Wang, B.J. Lorenzo, M.M. Reidenberg, NAD- and NADP-dependent 11 beta-hydroxysteroid dehydrogenase isoforms in guinea pig kidney with gossypol inhibition, Chung Kuo Yao Li Hsueh Pao 18 (6) (1997) 481–485.
[15] V. Lakshmi, C. Monder, Evidence for independent 11-oxidase and 11-reductase activities of 11 beta-hydroxysteroid dehydrogenase: enzyme latency, phase transitions, and lipid requirements, Endocrinology 116 (2) (1985) 552–560.
[16] A. Salva, G.R. Klinefelter, M.P. Hardy, Purification of rat Leydig cells: increased yields after unit-gravity sedimentation of collagenase-dispersed interstitial cells, J. Androl. 22 (2001) 665–671.
[17] A.H. Payne, J.R. Downing, K.L. Wong, Luteinizing hormone receptor and testosterone synthesis in two distinct populations of Leydig cells, Endocrinology 106 (1980) 1424–1429.
[18] R.S. Ge, H.B. Gao, V.L. Nacharaju, G.L. Gunsalus, M.P. Hardy, Identification of a kinetically distinct activity of 11β-hydroxysteroid dehydrogenase in rat Leydig cells, Endocrinology 138 (6) (1997) 2435–2442.
[19] A.G. Atanasov, L.G. Nashev, R.A. Schweizer, C. Frick, A. Odermatt, Hexose-6-phosphate dehydrogenase determines the reaction direction of 11β-hydroxysteroid dehydrogenase type 1 as an oxoreductase, FEBS Lett. 571 (1–3) (2004) 129–133.
[20] G. Banhegyi, A. Benedetti, R. Fulceri, S. Senesi, Cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum, J. Biol. Chem. 279 (26) (2004) 27017–27021.
[21] L. Duplomb, Y. Lee, M.Y. Wang, B.H. Park, K. Takaishi, A.K. Agarwal, R.H. Unger, Increased expression and activity of 11β-HSD-1 in diabetic islets and prevention with troglitazone, Biochem. Biophys. Res. Commun. 313 (3) (2004) 594–609.
[22] G.G. Lavery, E.A. Walker, N. Draper, P. Jeyasuria, J. Marcos, C.H. Shackleton, K.L. Parker, P.C. White, P.M. Stewart, Hexose-6-phosphate dehydrogenase knock-out mice lack 11 beta-hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation, J. Biol. Chem. 281 (10) (2006) 6546–6551.
[23] E.M. Coutinho, C. Athayde, G. Atta, Z.P. Gu, Z.W. Chen, G.W. Sang, E. Emuveyan, A.O. Adekunle, J. Mati, J. Otubu, M.M. Reidenberg, S.J. Segal, Gossypol blood levels and inhibition of spermatogenesis in men taking gossypol as a contraceptive. A multicenter, international, dose-finding study, Contraception 61 (1) (2000) 61–67.
[24] C. Dudel, U. Forstermann, Gossypol attenuates selectively the blood pressure lowering effect of endothelium-dependent vasodilators in the rabbit in vivo, Eur. J. Pharmacol. 145 (2) (1988) 217–221.