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Curcumin derivatives inhibit testicular 17β-hydroxysteroid dehydrogenase 3

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ABSTRACT

Non-steroidal compounds that inhibit 17β-hydroxysteroid dehydrogenase isoform 3 (17β-HSD3), an enzyme catalyzing the final step in testosterone biosynthesis in Leydig cells, are under development for male contraceptive or treatment of androgen dependent diseases including prostate cancer. A series of curcumin analogues with more stable chemical structures were compared to curcumin as inhibitors of 17β-HSD3 in rat intact Leydig cells as well as rat and human testis microsomes.

Inhibitors of testosterone (T) biosynthesis are widely sought for treatment of androgen-dependent disorders such as benign prostate hyperplasia, hormone-dependent prostate cancer, acne, seborrhea, hirsutism, androgenic alopecia, and precocious puberty and also for suppression of spermatogenesis as male contraceptives. The last step T biosynthesis from androstenedione is catalyzed by 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3).[1] This enzyme accounts for most of the circulatory T in males, as a genetic mutation of 17β-HSD3 causes the autosomal recessive genetic disorder male pseudohermaphroditism in which males often are born with female external genitalia and without a prostate.[2,3] Thus, drugs that inhibit 17β-HSD3 have potential for therapies where anti-androgenic action is desired.

The development of specific inhibitors of 17β-HSD3 and other 17β-HSD isoforms is the subject of a recent review.[4] These chemicals are: (1) androsterone (ADT) derivatives;[5–7] (2) biphenyl-*p*-benzoquinone (IC_{50} = 2.7 μM);[8] and (3) glycyrrhetinic acid.[9,10] ADT is a weak androgen that is twice as potent as androstenedione in the inhibition of 17β-HSD3.[5] A drawback of some of these derivatives such as 3β-phenylethyl-ADT (IC_{50} = 57 nM) is that they are also androgenic in the range of potencies similar to dihydrotestosterone.[5] Glycyrrhetinic acid also potently inhibits 11β-hydroxysteroid dehydrogenase 2 to cause hypertension.[11] We hypothesize that small molecules that block the active site of 17β-HSD3 inhibit the enzymatic activity. Therefore, we began to identify lead compounds from plant-derived phytochemicals. These nature chemicals generally are expected to have low toxicity and therefore could be potentially used for therapeutic use. Therefore, we screened many phytochemicals to inhibit 17β-HSD3. One of them is curcumin (Fig. 1), 1,7-bis(4-hydroxy-3-methoxypentyl)-1,6-hepadiene-3,5-dione, from turmeric. Recently, we synthesized many curcumin derivatives.[12] Then we screened these curcumin derivatives (Fig. 1) to inhibit rat and human 17β-HSD3 in intact rat Leydig cells, rat and human testis microsomes. The microsomal and cellular assay for 17β-HSD3 activity was performed.[13] The potencies of inhibiting 17β-HSD3 in rat intact Leydig cells, rat and human testis microsomes for curcumin were IC_{50} = 9.0 ± 1.0 (mean ± SEM, n = 4), 2.3 ± 1.2 (n = 3) and 67.3 ± 11.9 μM (n = 3), respectively. The inhibitory potencies of these curcumin derivatives are shown in Table 1. For the inhibition of human 17β-HSD3, the following chemicals were more potent than parent chemical curcumin: compound C3 (IC_{50} = 0.1 μM) > B4 (2.4 μM) > B6 (2.7 μM). However, these compounds were less potent to inhibit 17β-HSD3 in rat testis microsome. For example, IC_{50} of C3 was 1.8 μM for the inhibition of rat testis 17β-HSD3. There is species-dependent difference for the potencies of

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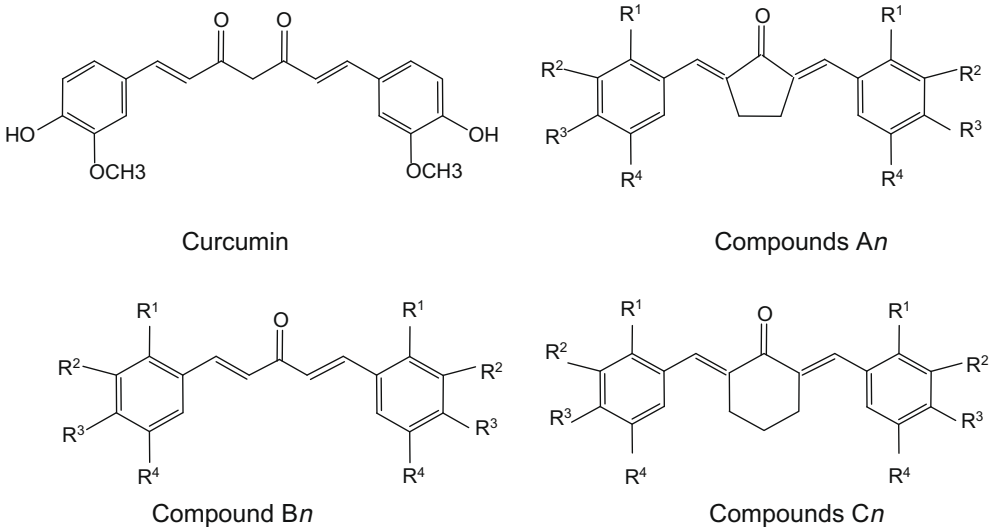

Compounds	R^1	R^2	R^3	R^4
A1, B1, C1	H	H	OCH2CH3	H
A2, B2, C2	H	H	F	H
A3, B3, C3	H	OCH3	OCH3	OCH3
A4, B4, C4	H	H	$N(CH_3)_2$	H
A6,B6,C6	H	Br	H	H
A7,B7,C7	H	H	O-m-C_5H_9O	H
A8,B8,C8	F	CF3	H	H
A10,B10,C10	H	Br	H	H
A11,B11,C11	H	OCH3	O-m-C_5H_9O	H
A12,B12,C12	H	OCH_3	-$OCH_2CH_2CH_2N(CH_3)_2$	H
A13,B13,C13	H	H	OH	H

Figure 1. Chemical structures of curcumin derivatives. *n* = carbon number in compounds A*n*, B*n* and C*n*.

Table 1
The inhibition of curcumin derivatives on 17β-hydroxysteroid dehydrogenase 3 in rat Leydig cells, rat and human testis microsome

A series	Rat		Human	B series	Rat		Human	C series	Rat		Human
	LCs	Testis	Testis		LCs	Testis	Testis		LCs	Testis	Testis
A1	14[a]	NI	NI	B1	(0.07)	16	16	C1	NI	16	13
A2	14	15	11	B2	(0.1)	(3.3)	39	C2	NI	NI	NI
A3	11	NI	NI	B3	NI	NI	NI	C3	(>100)	(1.8)	(0.1)
A4	NI	NI	NI	B4	NI	15	(2.4)	C4	12	10	17
A6	30	(0.7)[b]	14	B6	20	(8.5)	(2.7)	C6	25	40	15
A7	NI	13	NI	B7	(1.3)	29	22	C7	16	21	NI
A8	NI	23	NI	B8	–	–	–	C8	–	–	–
A10	NI	NI	NI	B10	–	–	–	C10	–	–	–
A11	NI	NI[c]	NI	B11	NI	35	20	C11	26	27	NI
A12	38	32	28	B12	–	–	–	C12	–	–	–
A13	18	15	28	B13	30	33	46	C13	13	34	38

Data were repeated twice with 200 nM androstenedione.
[a] % inhibition at 100 μM inhibitor.
[b] (IC_{50}, μM).
[c] NI = no inhibition; LCs = intact Leydig cells.

inhibition of the enzyme. For example, compound A6 was most potent in the inhibition of rat testis 17β-HSD3 (IC_{50} = 0.7 μM), while it only inhibited human enzyme by 14% at 100 μM. Some chemicals (e.g., C3) were less potent in intact rat Leydig cells compared to the testis microsome, indicating that these chemicals may not easily penetrate cell membrane.

Lineweaver–Burk plot analysis in the presence of 0, 1 and 10 μM curcumin with various concentrations of substrate androstenedione showed that curcumin was an uncompetitive inhibitor of both human and rat 17β-HSD3 (data not shown). This means that curcumin binds to the enzyme–substrate complex. The inhibition of luteinizing hormone stimulated T production in intact Leydig cells by curcumin was measured.[14] Curcumin significantly inhibited LH-stimulated T production when the concentration was at 10 μM (Fig. 2), indicating that curcumin can inhibit T production.

It was shown previously that curcumin can interact with several enzymes, but these interactions require relatively high con-

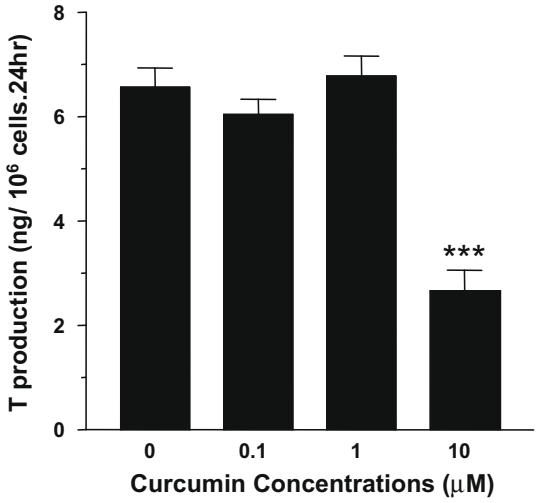

Figure 2. The effects of curcumin on LH-stimulated testosterone (T) production in adult Leydig cells (ALC). Different concentrations of curcumin (CUR) were incubated with 0.1×10^6 adult Leydig cells in the presence of 1 ng/ml LH and lipoproteins for 18 hrs. Mean ± SEM, $n = 8$, Asterisks '***' indicate the level of significance with a p value at 0.001 compared to control (without curcumin).

centrations (IC_{50} >10 μM) of curcumin.[15,16] In the present study, curcumin inhibited 17β-HSD3 with IC_{50} values of 2.3 μM (rat testis microsome). We also showed that 10 μM curcumin inhibited LH-stimulated T production to 36% of the control level (Fig. 2). Curcumin has been used as a coloring and flavoring additive in many foods, and the consumption in a normal diet is at the rate of up to 100 mg/day by people.[17] Human studies indicate that curcumin is tolerated in large oral doses, as high as to 8000 mg/day, without apparent toxicity.[18] Whether the inhibition of 17β-HSD3 can be achieved under normal dietary consumption requires further investigation. We discovered that compound C3 was very potent and inhibited human 17β-HSD3 with IC_{50} of 0.1 μM (Table 1), which was 673 times more potent than curcumin. Therefore, compound C requires further testing by in vivo animal models.

In conclusion, the present study showed that some curcumin derivatives are more potent than curcumin in the inhibition of human and rat 17β-HSD3 activities. There is apparently species-dependent difference for the potencies to inhibit 17β-HSD3.

Acknowledgments

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13. Adult rat Leydig cells were isolated from 90-day-old Sprague Dawley rat testes as previously described (Salva, A.; Klinefelter, G. R.; Hardy, M. P. *J. Androl.* **2001**, *22*, 665). Purities of Leydig cell were evaluated by histochemical staining for 3β-hydroxysteroid dehydrogenase with 0.4 mM etiocholanolone as the steroid substrate to show 95% purity (Payne, A. H.; Downing, J. R.; Wong, K. L. *Endocrinology* **1980**, *106*, 1424). The animal protocol was approved by the Institutional Animal Care and Use Committee of the Rockefeller University. Rat and human testis microsomes were prepared as described previously (Ge, R. S.; Gao, H. B.; Nacharaju, V. L.; Gunsalus, G. L.; Hardy, M. P. *Endocrinology* **1997**, *138*, 2435). The activity of 17β-HSD3 in intact Leydig cells or microsomes. 0.05×10^6 cells or 40–50 μg human or rat testis microsomes were incubated with 200 nM ^{3}H-androstenedione (Dupont-New England Nuclear, Boston, MA) and 0.2 mM NADPH (for microsomes only) for 1.5–2 h. Steroids were separated by thin layer chromatography and scanned by a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC). Various concentrations of curcumin (Sigma–Aldrich, St. Louis, MO) and its derivatives (synthesized as previously described) were used.
14. 0.1×10^6 Leydig cells were cultured for 24 h in DMEM:F12 with various concentrations of curcumin in the absence or presence of 1 ng/ml LH. T present in medium was measured by a tritium-based radioimmunoassay (RIA).
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