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Systematic Trial for Evaluating Docetaxel in a Human Prostate Cancer Cell DU145 Xenograft Model

TOSHIDA =>YOSHIDA HORIIKE KOTA=> KOTA HORIIKE

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Abstract. *The inhibitory activities of docetaxel at a wide range of doses (0.1-10 mg/kg; subcutaneously (s.c.), once/week) in nude mice bearing a human prostate cancer cell, DU145, xenograft model with implantation of DU145 cells or a DU145 solid tumor were examined. This systematic trial demonstrated that (i) docetaxel was more effective in the xenograft model formed by implantation of DU145 cells than in the solid DU145 tumor; (ii) administration of 2.5 mg/kg docetaxel was the critical dose because inhibitory activities were not observed at 2.5 mg/kg, while they were noted at 5 mg/kg and 10 mg/kg in both implantation approaches; (iii) edema-like effects (plump body shape and legs) were observed in both groups at 2.5 mg/kg and the tumor sizes, often increased by blood plasma and other fluids, as well as body weights were higher than at other doses; and (iv) suppression of body weight gain was observed at 10 mg/kg docetaxel.*

Prostate cancer is one of the leading causes of male mortality due to cancer and its prevalence is now increasing; therefore, the development of effective new drugs is now required (1). Androgen ablation therapy is usually effective for this cancer at an earlier stage because the tumors typically depend on androgen for development and growth at that period. However, alternative drugs are needed to treat androgen-independent tumors (2). Docetaxel (DTX) is a taxane derivative, widely used anti-microtubule anti-cancer drug, and an effective chemotherapeutic drug for first-line treatment of hormone-refractory prostate cancer (3). Therefore, combination and comparison studies *in vitro* and *in vivo* with DTX are vital for developing novel anti-prostate cancer agents.

DU145 is a human hormone-independent prostate cancer cell line widely used for evaluating novel anti-prostate cancer agents (4). The inhibitory activity of DTX against DU145 *in vitro* exhibited a half maximal inhibitory concentration (IC_{50}) of 1.1 nM (Figure 1). A xenograft model of DU145 tumor growth in nude mice is useful for evaluating agents that can treat hormone-refractory prostate cancer. However, the dose-dependence of DTX in such xenograft mice has not been reported, even though it is vital for the screening and development of novel anti-prostate cancer drugs. We report the results of a systematic trial on growth suppression of implanted DU145 tumors and side-effects with DTX in a wide range (0.1-10 mg/kg; subcutaneously (*s.c.*); in a week) in two models, a xenograft model formed by implantation of a mixture of DU145 cells and Matrigel™ and a model formed by implantation of solid DU145 tumors (Figure 2).

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Key Words: Docetaxel, DU145, xenograft model, nude mice, anti-cancer drug, dose-dependency, side-effects.

Materials and Methods

Cell line and cell culture. The human prostate cancer cell line DU145 was supplied from Riken BioResource Center (#RCB2143), Tsukuba, Japan. The cell line was maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin at 37˚C in a humidified atmosphere containing 5% CO_2.

Inhibition of DU145 growth in vitro by DTX. DTX (#D4102) was purchased from Tokyo Chemical Industry Co., Ltd. Tokyo, Japan. DU145 was seeded at a density of 1,700 cells/ well on 96-well plates (Sumitomo Bakelite Co., Ltd., Tokyo, Japan). The next day, DTX was added to each well at concentrations of 0, 0.4, 0.6, 0.8, 1 and 2 nM (final in 0.1% DMSO). After 3 days, the numbers of viable cells

were determined using WST-1 (#W201; 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) and 1-methoxy-5-methylphenazinium methylsulfate (#M003), which were purchased from Dojindo Laboratories (Kumamoto, Japan). After the addition of WST-1, the absorbance was measured at 450 nm with 630 nm as a reference wavelength using a Versa Max reader (Molecular Devices, Tokyo, Japan).

Xenograft model formed by implantation of a mixture of DU145 cells and Matrigel™ (Figure 2A). Male nude BALB/c mice were purchased from Japan SLC Inc. (Shizuoka, Japan). The animals were housed in constant temperature and humidity conditions and fed a standard diet with water *ad libitum*. Our institutional animal care committee approved all animal experiments. Male nude mice were subcutaneously implanted with approximately 100 μl of 8×10^6 DU145 cells mixed with Matrigel™ Basement Membrane Matrix high Concentration (Matrigel™) (#354248; Corning, Tokyo, Japan) into the right flank. When the estimated tumor volume reached approximately 200 mm^3, mice bearing established tumors were randomized into five treatment groups (vehicle; 1, 2.5, 5 and 10 mg/kg, DTX; n=6 for each group) with an almost equivalent average body weight. The mice were subcutaneously treated with each concentration of DTX in PEG300 once per week and observed for 26 days. The tumor volumes (V) were calculated by caliper measurements of the width (W) and length (L) of each tumor using the formula: V=W×W×L/2.

Xenograft model formed by implantation of a solid tumor of DU145 (Figure 2B). Male nude mice were subcutaneously implanted with approximately 100 μl of 8×10^6 DU145 cells mixed with Matrigel™ into the right flank. The tumor was obtained from the mice and split into pieces (particle sizes of approximately 3 mm × 3 mm × 3 mm) and then subcutaneously implanted into 5-week-old nude mice. When the estimated tumor volume reached ca. 100 mm^3, mice bearing established tumors were randomized into 8 treatment groups (vehicle; 0.1, 0.5, 0.75, 1.0, 2.5, 5.0 and 10 mg/kg DTX; n=5 for each group) with an almost equivalent average of body weight and tumor volumes. The mice were subcutaneously treated with each concentration of DTX in 0.5% methylcellulose (MC) for 32 days once per week. Tumor volumes and body weight were periodically measured. Animals were sacrificed after 32 days and the weights of the tumor, liver, kidney and spleen were measured. Blood was collected from the abdominal vein with heparin as an anticoagulant and the plasma was obtained by centrifugation. The concentrations of serum glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), creatinine and blood urea nitrogen (BUN) were measured using commercially available kits (Transaminase CII Test WAKO #431-30901, LabAssay Creatinine #290-65901, L type WAKO UN #410-55391 #416-55951; WAKO Pure Chemical Industries, Ltd., Osaka, Japan).

Statistical analysis. The results are shown as the means±standard error (SE) of each group and were analyzed for statistical significance using Student's *t*-test compared with vehicle control.

Results

Inhibition of DU145 growth in vitro by DTX. The inhibitory activity of DU145 cell growth by DTX was estimated (Figure 1). The IC_{50} value was 1.1 nM in this study after 72 h of drug exposure, which was approximately in agreement with a previous report's IC_{50} values of 0.47 nM (4) and 1.1 nM (5).

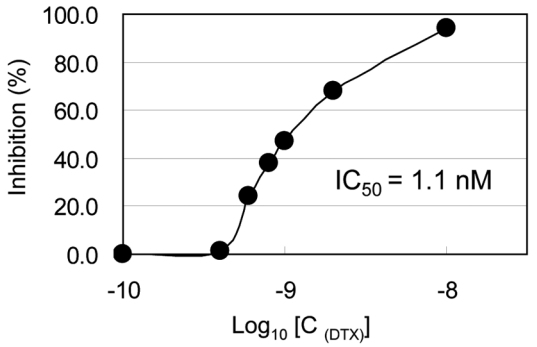

Figure 1. *Inhibition of DU145 growth by docetaxel in vitro. DTX, docetaxel; IC_{50}, half maximal inhibitory concentration.*

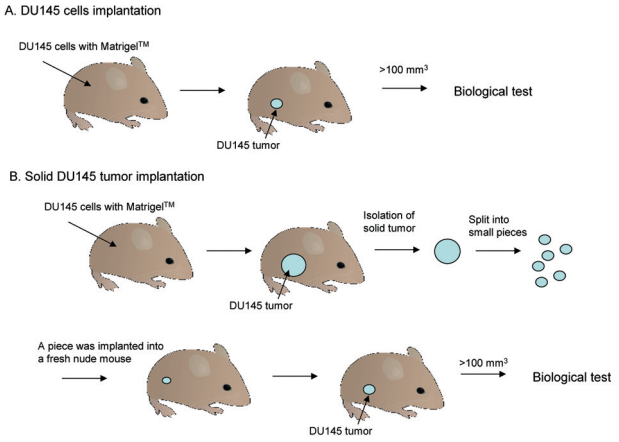

Figure 2. *The two xenograft models evaluated in this study.*

Suppression of xenograft DU145 tumor by DTX. There were two xenograft murine models bearing DU145 cells (Figure 2). First, the inhibitory effect of DTX on DU145 tumor growth after subcutaneous implantation with DU145 cells mixed with Matrigel™ was examined (Figure 2A). Vehicle or DTX (1, 2.5, 5 and 10 mg/kg) was subcutaneously administered once a week for 26 days and the tumor volume (mm^3), as well as body weight (g), were periodically measured (Figure 3; Tables I, II and III). The tumor growth was slower than control growth at doses of 1.0, 5.0 and 10 mg/kg DTX, though tumor growth was accelerated at 2.5 mg. Tumor sizes at 10 mg/kg DTX were significantly reduced within a few days ($p<0.05$ by Student's *t*-test). Interestingly, tumors in the 2.5 mg/kg DTX group were often filled with liquid matter, such as blood plasma and sometimes blood, while tumors at the other doses consisted of cells (Figure 3D). The body weights of mice were reduced

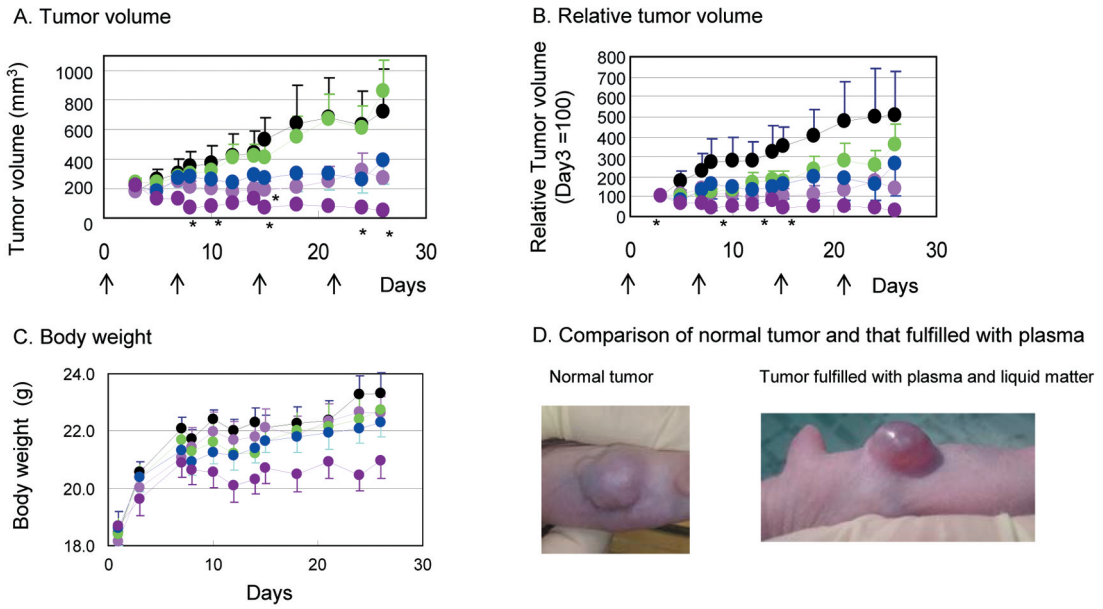

Figure 3. *Suppression of DU145 cells growth in vivo by docetaxel. Male nude mice were subcutaneously implanted with approximately 8×10^6 DU145 cells mixed with Matrigel™ into the right flank. When the estimated tumor volume reached almost 200 mm^3, docetaxel was subcutaneously administered once a week after randomization into 5 treatment groups (black: vehicle, pink: 1, green: 2.5, blue: 5 and purple: 10 mg/kg docetaxel, n=6 for each group). A: Tumor volumes were calculated by caliper measurements of the width (W) and length (L) (Volume=W×W×L/2). B: Relative tumor volume (volumes at day 3 are 100). C. Body weights. D. Edematous phenomena in tumors were observed with 2.5 mg/kg docetaxel. All values were tabulated in Tablew I-III. Each value represents the mean±SE of six mice. *p<0.05 comparison with control by Student's t-test.*

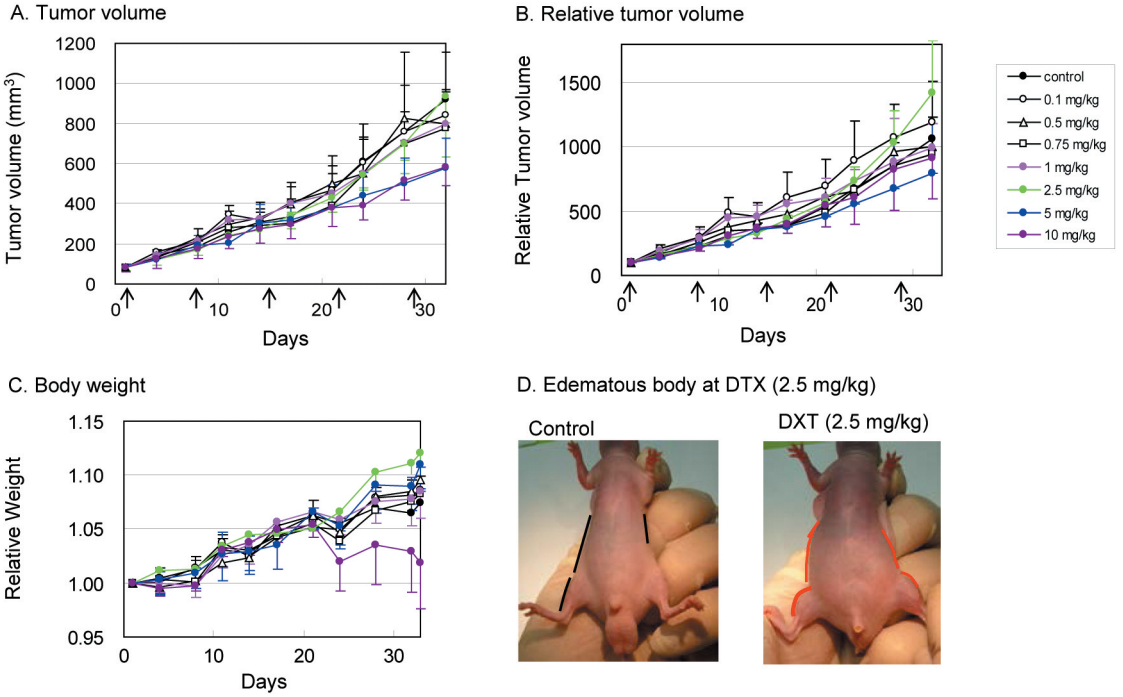

Figure 4. *Suppression of DU145 cell block growth in vivo by docetaxel. Male nude mice were subcutaneously implanted with solid tumor into the right flank. When the estimated tumor volume reached almost 100 mm3, docetaxel was subcutaneously administered once a week after randomization into 8 treatment groups (black closed circle: vehicle, black open circle: 0.1 mg/kg, black open triangle: 0.5 mg/kg, black open square: 0.75 mg/kg, pink:1, green: 2.5, blue: 5 and purple: 10 mg/kg docetaxel, n=6 for each group). A: Tumor volumes were calculated by caliper measurements of the width (W) and length (L) (volume=W×W×L/2). B: Relative tumor volume (Volume at day 1=100). C: Body weight. D: Edematous phenomena were observed in mice with 2.5 mg/kg docetaxel. All values were tabulated in Tables I-III. Each value represents the mean±SE of five mice.*

Table Ia. *Tumor size (mm^3), averaged tumor size and standard error (SE) values.*

Dosage	Animal No.	Days											
		3	5	7	8	10	12	14	15	18	21	24	26
Vehicle	1	35.0	130.6	228.7	299.4	295.3	232.2	323.4	270.1	327.7	484.1	586.625	529.83
	2	125.4	247.2	207.6	241.8	320.6	458.6	468.5	517.6	420.2	458.6	386.6	403.5
	3	277.3	261.8	480.3	588.1	616.5	839.8	836.4	1127.1	1822.5	1822.5	1565.0	1909.3
	4	121.3	238.3	149.4	135.0	118.0	0.0	0	245.1	184.6	78.0	110.9	143.7
	5	352.5	583.2	689.1	740.1	807.4	878.4	870.9	849.4	957.6	1112.9	1029.6	1233.9
	6	171.2	85.2	74.0	81.9	54.7	127.1	142.9	144.9	107.2	98.8	71.5	78.8
	Average	180.4	257.7	304.8	347.7	368.7	422.7	440.4	525.7	636.6	675.8	625.0	716.5
	SE	47.2	71.3	95.1	106.6	118.7	151.1	146	158.7	266.9	275.7	236.4	292.0
DTX	7	151.2	133.2	180.8	81.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1 mg/kg	8	257.1	163.3	325.8	239.6	250.0	249.7	256.6	259.2	301.2	443.8	455.2	490.4
	9	151.2	263.8	316.9	254.6	365.1	158.8	200.9	154.9	202.0	155.7	250.3	0.0
	10	162.5	174.6	221.4	202.6	198.6	292.5	282.0	371.3	386.9	502.2	683.6	619.5
	11	194.4	290.4	372.0	346.4	292.5	310.3	296.5	337.9	372.0	427.6	533.0	507.8
	12	165.3	154.7	100.0	143.0	83.9	70.7	98.8	0.0	0.0	0.0	0.0	0.0
	Average	180.3	196.7	252.8	211.3	198.3	180.3	189.1	187.2	210.4	254.9	320.3	269.6
	SE	16.7	26.3	42.1	37.6	55.3	51.4	47.9	66.6	71.7	94.2	116.3	121.9
DTX	13	328.1	292.5	249.2	309.3	346.8	470.5	386.6	414.1	506.0	738.1	542.6	724.7
2.5 mg/kg	14	245.5	205.8	346.8	406.5	341.2	358.4	363.4	392.1	597.9	677.2	608.6	1106.6
	15	203.7	253.6	295.3	246.1	239.6	409.6	476.6	407.2	636.3	630.6	649.4	864.6
	16	209.1	259.6	417.9	503.6	576.0	778.5	769.2	893.8	1069.2	1351.7	1224.9	1580.1
	17	171.0	196.6	103.2	113.8	89.9	73.6	180.8	88.9	27.6	40.3	69.8	36.1
	18	293.5	233.3	287.6	226.8	303.2	372.0	358.4	271.1	477.9	577.4	559.8	844.0
	Average	241.8	240.2	283.3	301.0	316.1	410.4	422.4	411.2	552.5	669.2	609.2	859.4
	SE	24.3	14.6	43.2	56.5	64.9	92.5	79.7	109.1	136.5	170.9	150.4	206.3
DTX	19	196.5	272.0	194.5	200.7	250.5	257.4	252.3	211.3	247.6	245.2	267.1	353.0
5 mg/kg	20	98.4	95.0	280.9	502.2	406.8	359.7	346.6	525.1	734.5	715.5	534.6	1026.7
	21	316.0	257.4	317.2	271.8	144.5	146.2	270.7	133.2	50.4	137.9	95.0	148.8
	22	280.8	361.3	520.7	541.0	509.9	523.4	520.7	486.0	567.6	604.7	482.4	688.2
	23	261.8	104.0	195.3	81.8	106.4	73.7	175.7	197.6	70.0	58.7	90.4	132.9
	24	191.5	0.0	105.9	102.5	158.9	74.1	158.1	93.3	108.0	63.5	81.6	0.0
	Average	224.2	181.6	269.1	283.3	262.8	239.1	287.4	274.4	296.3	304.3	258.5	391.6
	SE	32.0	55.6	58.7	80.5	66.1	72.8	54.4	75.3	117.6	116.7	84.2	160.4
DTX	25	409.9	170.7	242.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10 mg/kg	26	217.7	153.0	103.9	182.6	153.1	200.4	236.9	138.9	143.7	81.3	109.8	0.0
	27	124.7	80.0	133.2	88.8	88.8	106.8	186.4	55.6	68.7	96.1	91.0	76.0
	28	180.3	160.7	134.0	110.5	76.3	80.0	95.6	102.6	107.6	99.7	65.7	80.1
	29	263.5	139.2	136.6	0.0	86.8	131.2	184.0	88.8	155.7	156.6	79.2	71.9
	30	93.9	59.2	59.1	52.8	65.6	75.0	99.1	58.1	62.4	66.7	66.8	46.2
	Average	215.0	127.1	134.9	72.4	78.4	98.9	133.7	74.0	89.7	83.4	68.7	45.7
	SE	46.3	18.9	24.7	28.7	20.1	27.2	34.9	19.4	23.7	20.8	15.3	15.2

Table Ib. *Student's t-test (vs. vehicle).*

T-TEST *vs.* Vehicle	Days											
	3	5	7	8	10	12	14	15	18	21	24	26
DTX (1 mg/kg)	0.998	0.440	0.628	0.255	0.223	0.160	0.133	0.078	0.154	0.179	0.274	0.188
DTX (2.5 mg/kg)	0.274	0.815	0.841	0.707	0.706	0.946	0.917	0.565	0.785	0.984	0.956	0.698
DTX (5 mg/kg)	0.460	0.419	0.756	0.640	0.454	0.299	0.349	0.183	0.270	0.243	0.175	0.352
DTX (10 mg/kg)	0.612	0.107	0.114	0.032	0.037	0.061	0.068	0.018	0.068	0.058	0.041	0.045

$p<0.05$

almost dose-dependently, except with the administration of 2.5 mg of DTX; significance was observed in the 10 mg/kg group compared with control.

Next, the inhibitory activities of tumor growth with DTX in xenograft mice after implantation of solid DU145 tumor was examined (Figure 2B). Vehicle or DTX at a wide range

Table IIa. *Relative tumor size (Day 3=100), averaged tumor size and standard error (SE) values.*

Dosage	Animal No.	Days											
		3	5	7	8	10	12	14	15	18	21	24	26
Vehicle	1	100	373	654	856	844	664	924	772	936	1383	1676	1514
	2	100	197	166	193	256	366	374	413	335	366	308	322
	3	100	94	173	212	222	303	302	407	657	657	564	689
	4	100	197	123	111	97	0	0	202	152	64	91	119
	5	100	165	195	210	229	249	247	241	272	316	292	350
	6	100	50	43	48	32	74	83	85	63	58	42	46
	Average	100	179	226	272	280	276	322	353	403	474	496	507
	SE		46	88	120	118	96	133	98	135	203	248	221
DTX	7	100	88	120	54	0	0	0	0	0	0	0	0
1 mg/kg	8	100	64	127	93	97	97	100	101	117	173	177	191
	9	100	174	210	168	241	105	133	102	134	103	166	0
	10	100	108	136	125	122	180	174	229	238	309	421	381
	11	100	149	191	178	151	160	153	174	191	220	274	261
	12	100	94	61	86	51	43	60	0	0	0	0	0
	Average	100	113	141	117	110	97	103	101	113	134	173	139
	SE		17	22	20	34	28	26	37	40	50	66	67
DTX	13	100	89	76	94	106	143	118	126	154	225	165	221
2.5 mg/kg	14	100	84	141	166	139	146	148	160	244	276	248	451
	15	100	124	145	121	118	201	234	200	312	310	319	424
	16	100	124	200	241	275	372	368	427	511	646	586	756
	17	100	115	60	67	53	43	106	52	16	24	41	21
	18	100	79	98	77	103	127	122	92	163	197	191	288
	Average	100	103	120	128	132	172	183	176	233	280	258	360
	SE		8	21	27	31	45	42	54	69	84	76	101
DTX	19	100	138	99	102	127	131	128	108	126	125	136	180
5 mg/kg	20	100	97	285	510	413	365	352	533	746	727	543	1043
	21	100	81	100	86	46	46	86	42	16	44	30	47
	22	100	129	185	193	182	186	185	173	202	215	172	245
	23	100	40	75	31	41	28	67	75	27	22	35	51
	24	100	0	55	54	83	39	83	49	56	33	43	0
	Average	100	81	133	163	149	133	150	163	196	194	160	261
	SE		22	35	73	57	53	44	77	114	111	80	161
DTX	25	100	42	59	0	0	0	0	0	0	0	0	0
10 mg/kg	26	100	70	48	84	70	92	109	64	66	37	50	0
	27	100	64	107	71	71	86	150	45	55	77	73	61
	28	100	89	74	61	42	44	53	57	60	55	36	44
	29	100	53	52	0	33	50	70	34	59	59	30	27
	30	100	63	63	56	70	80	106	62	66	71	71	49
	Average	100	64	67	45	48	59	81	43	51	50	44	30
	SE		7	9	15	12	14	21	10	10	11	11	11

Table IIb. *Student's t-test (vs. vehicle).*

T-TEST *vs.* Vehicle	Days											
	3	5	7	8	10	12	14	15	18	21	24	26
DTX (1 mg/kg)	-	0.201	0.372	0.233	0.198	0.104	0.138	0.037	0.068	0.136	0.237	0.143
DTX (2.5 mg/kg)	-	0.129	0.272	0.268	0.254	0.351	0.342	0.146	0.291	0.397	0.381	0.561
DTX (5 mg/kg)	-	0.079	0.355	0.455	0.341	0.221	0.249	0.158	0.269	0.255	0.226	0.390
DTX (10 mg/kg)	-	0.031	0.104	0.090	0.079	0.049	0.105	0.011	0.027	0.064	0.098	0.057

$p<0.05$

of doses (0.1, 0.5, 0.75, 1, 2.5, 5 and 10 mg/kg) were subcutaneously administered once a week for 32 days and the tumor volume and body weights were measured (Figure 4; Tables IV, V and VI). Tumor sizes were reduced by subcutaneous administration of DTX at 5.0 and 10 mg/kg, while those at 0.1, 0.5, 0.75, 1.0 and 2.5 mg/kg did not have

Table IIIa. *Body weights (g) and standard error (SE) values.*

Dosage	Animal No.	Days											
		3	5	7	8	10	12	14	15	18	21	24	26
Vehicle	1	15.9	20.7	22.0	21.6	22.6	22.2	22.6	22.7	22.8	23.4	23.8	23.9
	2	18.0	20.1	21.6	21.2	21.9	21.7	21.7	21.4	21.2	20.6	21.8	21.2
	3	17.7	20.3	22.4	22.5	23.1	23.4	24.0	23.4	24.7	24.7	26.1	26.0
	4	19.9	21.3	23.4	22.6	23.2	22.8	23.0	22.9	22.2	22.6	23.3	23.2
	5	21.0	21.8	22.6	22.0	22.5	21.6	22.2	21.8	22.3	22.6	23.0	24.0
	6	18.2	19.2	20.6	20.4	21.2	20.4	20.1	20.5	20.3	20.3	21.6	21.6
	Average	18.5	20.6	22.1	21.7	22.4	22.0	22.3	22.1	22.3	22.4	23.3	23.3
	SE	0.7	0.4	0.4	0.3	0.3	0.4	0.5	0.4	0.6	0.7	0.7	0.7
DTX	7	16.4	18.5	19.2	20.2	20.9	20.8	21.2	21.2	21.2	21.3	21.8	22.0
1 mg/kg	8	17.6	19.4	21.0	21.6	22.1	22.2	22.2	21.9	21.2	21.9	22.2	22.6
	9	18.6	20.8	22.6	22.8	23.4	22.8	22.6	23.3	23.2	23.6	23.5	22.0
	10	19.6	21.1	21.0	21.0	21.5	21.2	21.5	22.2	22.2	22.3	22.3	23.0
	11	19.8	22.3	23.4	23.8	24.3	23.8	23.6	24.4	24.0	24.5	25.2	25.2
	12	16.8	18.0	19.2	19.2	19.7	19.4	19.4	19.8	19.4	20.5	21.0	20.9
	Average	18.1	20.0	21.1	21.4	22.0	21.7	21.8	22.1	21.9	22.4	22.7	22.6
	SE	0.6	0.7	0.7	0.7	0.7	0.6	0.6	0.7	0.7	0.6	0.6	0.6
DTX	13	16.5	18.6	20.0	19.4	19.7	18.8	19.4	19.6	19.4	20.0	19.5	20.4
2.5 mg/kg	14	18.0	19.1	21.2	20.4	21.0	21.0	21.8	21.8	22.0	22.0	22.4	22.9
	15	17.9	20.1	22.2	21.6	22.3	21.8	22.4	22.6	22.2	22.3	23.0	23.4
	16	18.8	21.5	22.2	22.0	22.5	22.2	21.6	22.8	24.4	24.4	24.6	25.1
	17	19.8	22.0	23.2	23.0	23.0	23.0	22.0	22.6	22.8	23.0	24.0	23.8
	18	19.3	21.0	21.4	21.4	21.2	20.4	20.2	20.6	21.2	21.3	20.9	20.9
	Average	18.4	20.4	21.7	21.3	21.6	21.2	21.2	21.7	22.0	22.2	22.4	22.8
	SE	0.5	0.6	0.4	0.5	0.5	0.6	0.5	0.5	0.7	0.6	0.8	0.7
DTX	19	16.7	19.0	19.8	19.6	20.2	19.6	19.4	20.0	19.8	19.9	20.1	20.2
5 mg/kg	20	17.9	19.0	20.2	19.6	19.8	19.8	20.6	21.0	21.3	21.2	21.5	21.7
	21	18.1	20.2	21.6	21.2	21.8	21.8	22.0	22.4	22.0	21.8	22.2	22.7
	22	18.3	20.6	21.2	21.0	21.4	21.2	21.8	21.4	21.4	21.6	21.9	22.1
	23	20.8	22.0	22.8	22.4	22.2	22.4	22.4	22.6	22.8	23.3	23.4	23.4
	24	19.9	21.6	22.4	21.8	22.2	22.0	22.2	22.6	23.4	23.8	23.4	23.8
	Average	18.6	20.4	21.3	20.9	21.3	21.1	21.4	21.7	21.8	21.9	22.1	22.3
	SE	0.6	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.6	0.5	0.5
DTX	25	17.3	18.6	20.0	19.4	19.0	18.6	18.6	18.8	18.4	18.8	18.4	18.4
10 mg/kg	26	18.2	18.8	20.2	20.2	20.4	19.6	20.0	20.4	20.4	20.7	20.8	21.4
	27	17.7	19.0	20.8	20.8	20.6	20.4	20.6	21.6	21.6	21.4	20.8	21.2
	28	19.3	20.2	20.8	20.6	20.8	19.8	20.0	20.2	20.0	20.6	19.8	20.4
	29	21.6	22.4	23.4	23.0	23.0	22.8	22.6	22.6	22.8	23.0	22.6	23.0
	30	18.0	18.8	20.2	19.8	19.6	19.4	20.0	20.6	19.8	21.0	20.4	21.4
	Average	18.7	19.6	20.9	20.6	20.6	20.1	20.3	20.7	20.5	20.9	20.5	21.0
	SE	0.6	0.6	0.5	0.5	0.6	0.6	0.5	0.5	0.6	0.6	0.6	0.6

Table IIIb. *Student's t-test (vs. vehicle).*

T-TEST *vs.* Vehicle	Days											
	3	5	7	8	10	12	14	15	18	21	24	26
DTX (1 mg/kg)	-	0.800	0.674	0.759	0.683	0.618	0.516	0.573	0.425	0.382	0.275	0.237
DTX (2.5 mg/kg)	-	0.782	0.725	0.676	0.581	0.504	0.408	0.468	0.401	0.335	0.249	0.255
DTX (5 mg/kg)	-	0.764	0.628	0.595	0.490	0.446	0.382	0.410	0.308	0.245	0.166	0.158
DTX (10 mg/kg)	-	0.709	0.618	0.601	0.449	0.353	0.279	0.315	0.223	0.185	0.086	0.092

a obvious effect on the tumor size (Figure 4A and 4B). Tumors filled with liquid were also observed in mice in the 2.5 mg/kg DTX group. Growth suppression of the body weight in the 10 mg/kg DTX group was also observed (Figure 4C). It is known that clinical use of DTX can cause edema (6); similar phenomena were observed in mice at the

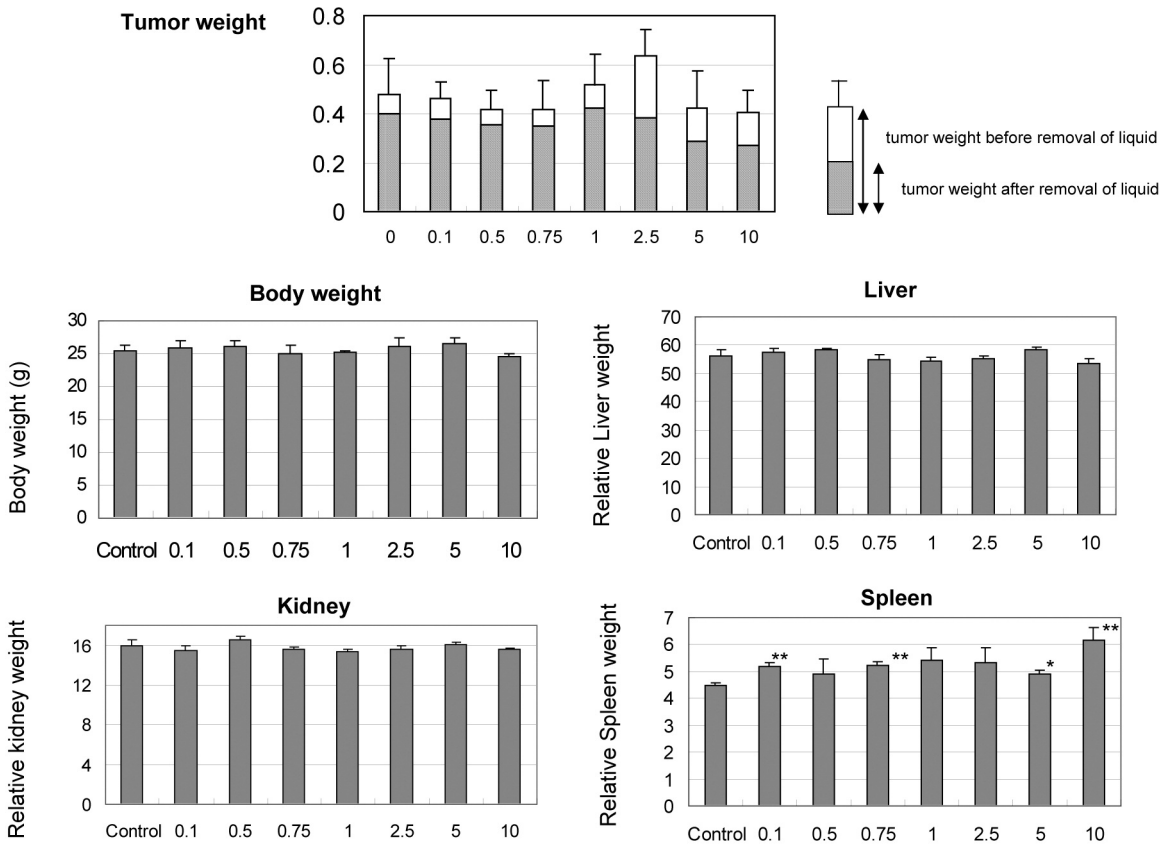

Figure 5. *Average of the relative weights of the tumor, body weight, liver, kidneys and spleen. Each value represents the mean±SE of five mice. * and **indicate p<0.05 and 0.01 compared to control with Student's t-test. Each relative weight was estimated by the following formula: (relative organ weight) (g/kg) =(weight of organs) (g) / (body weight) (kg).*

2.5 mg/kg dose of DTX (Figure 4D). In that group, almost 40% of the tumor weight consisted of liquid matter (Figure 5) and significance was observed in the fluid weight of the tumor compared with control. To estimate the toxicity with the administration of DTX, the weights of body, liver, kidneys and spleen were measured on the final day; they were nearly at the control levels (Figure 5). Hypertrophy of the spleen was frequently noted at all conditions, while dose-dependence was unclear. The concentrations of plasma GOT, GPT, creatinine and BUN on the final day were also estimated. GOT and GPT values were significantly low, except for at 0.1 mg/kg (Figure 6). BUN and creatinine were somewhat increased, albeit not dose-dependently.

Discussion

Inhibitory activities of DTX in DU145 xenograft mice were examined under two conditions (Figure 2A and 2B). DTX was subcutaneously injected for 26 days (Figure 3; Tables I, II and III) or 32 days (Figure 4; Tables IV, V and VI), once per week, and the tumor volume and body weights were measured. In previously reported works, administration of 25 mg/kg DTX (intravenously (*i.v.*), 5 days/week, 6 weeks) completely suppressed DU145 tumor growth in a xenograft model (7), while, intraperitoneally (*i.p.*), at 10 mg/kg (once/week, 3 weeks), DTX partially inhibited the growth of DU145 tumors in a xenograft model (8). Moreover, there are similar reports at a dose higher than 15 mg/kg in an interview (Sanofi Co. Ltd.) (5) and over 8.9 mg/kg elsewhere (9). However, DTX is clinically used at a dose of almost 2-3 mg/kg (75 mg/m2) by infusion over 3 hours once every 3 weeks in Japan (5). According to our search, this is the first report to examine the dose-dependence of DTX at a wide range of doses on the inhibition of tumor growth in xenograft mice bearing DU145 tumors. DTX at 1, 5 and 10 mg/kg doses inhibited tumor growth in a xenograft model that was developed by the injection of DU145 cells (Figure 3). However, suppression of tumor growth was not observed

Table IVa. *Tumor size (mm^3), averaged tumor size and standard error (SE) values.*

Dosage	Animal No.	Days									
		1	4	8	11	14	17	21	24	28	32
Vehicle	31	53.7	54.2	114.9	89.2	114.4	134.54	159.0	201.6	276.8	397.8
	32	62.4	122.4	143.4	198.5	230.6	235.20	277.2	290.5	399.4	548.4
	33	84.7	159.5	198.5	321.5	325.1	276.82	428.1	418.2	494.0	670.7
	34	94.9	213.6	269.8	328.1	414.7	422.33	665.5	984.4	1201.3	1481.1
	35	111.3	177.3	212.7	365.1	469.8	617.98	812.3	1149.2	1430.7	1503.4
	Mean	81.4	145.4	187.8	260.5	310.9	337.37	468.4	608.8	760.4	920.3
	SE	10.5	27.1	27.2	51.2	63.8	84.03	120.6	191.9	232.2	237.5
DTX	36	50.4	146.9	303.2	449.6	429.0	673.67	716.6	1027.0	930.2	1090.4
0.1 mg/kg	37	66.7	126.9	132.1	233.4	306.6	367.84	447.2	532.5	652.9	670.0
	38	71.4	175.7	220.3	450.6	370.9	465.75	578.7	675.5	1046.9	1211.0
	39	106.0	189.6	193.0	303.4	272.3	283.97	284.8	358.7	562.3	557.0
	40	112.1	158.8	231.9	292.0	213.4	244.49	322.5	426.3	612.9	672.3
	Mean	81.3	159.6	216.1	345.8	318.4	407.14	469.9	604.0	761.0	840.1
	SE	11.9	10.9	27.8	44.2	37.6	76.70	80.4	118.5	95.8	129.9
DTX	41	46.6	118.6	239.7	332.8	401.2	358.40	480.3	495.1	622.4	758.2
0.5 mg/kg	42	57.6	75.8	95.6	81.6	97.5	111.39	173.4	171.1	279.3	382.3
	43	86.0	114.9	190.8	241.9	213.6	242.59	368.3	336.0	483.8	649.0
	44	103.9	223.8	251.4	425.0	494.1	496.86	472.4	486.7	645.0	839.8
	45	117.0	171.5	379.5	418.2	446.6	763.99	1000.8	1254.5	2107.5	1362.1
	Mean	82.2	140.9	231.4	299.9	330.6	394.65	499.0	548.7	827.6	798.3
	SE	13.4	25.7	46.1	63.9	75.2	112.15	137.1	186.1	326.5	160.7
DTX	46	44.4	50.3	127.0	123.6	139.4	152.46	168.8	213.2	287.1	202.6
0.75 mg/kg	47	62.4	136.5	194.5	295.9	336.0	389.34	511.5	612.1	772.8	1089.3
	48	81.1	124.5	161.4	218.1	196.5	178.18	204.7	312.1	362.4	447.2
	49	102.7	181.3	292.0	439.6	461.7	459.60	615.3	1086.5	1421.6	1415.8
	50	117.2	181.5	262.1	329.3	313.6	340.22	443.6	488.4	640.5	717.3
	Mean	81.6	134.8	207.4	281.3	289.4	303.96	388.8	542.5	696.9	774.4
	SE	13.2	24.1	30.7	53.1	56.4	59.83	87.1	152.5	201.7	217.7
DTX	51	45.5	101.9	170.6	245.5	233.3	340.61	303.2	414.3	348.8	404.6
1 mg/kg	52	57.3	102.8	222.0	426.5	481.6	545.72	590.0	646.9	792.6	837.8
	53	81.6	148.1	254.9	249.4	270.7	285.91	358.7	342.2	363.4	426.5
	54	99.8	188.5	188.5	356.4	320.6	382.34	473.1	700.2	1118.7	1156.6
	55	120.9	180.9	243.7	323.8	317.5	450.64	521.3	646.9	886.1	1166.9
	Mean	81.0	144.4	215.9	320.3	324.7	401.04	449.3	550.1	701.9	798.5
	SE	13.7	18.5	16.0	34.1	42.4	45.08	52.5	71.7	150.9	167.2
DTX	56	35.6	97.5	108.8	97.5	119.1	202.50	282.1	402.2	702.3	1067.7
2.5 mg/kg	57	54.4	61.9	102.5	144.3	181.8	234.48	290.5	423.4	605.4	773.3
	58	76.7	82.5	181.3	181.7	262.8	293.49	422.7	494.1	612.6	769.8
	59	109.8	189.2	237.1	335.2	292.8	424.13	483.8	582.6	566.2	682.3
	60	133.0	176.1	230.7	452.8	480.3	549.21	656.7	822.0	1021.5	1390.6
	Mean	81.9	121.5	172.1	242.3	267.4	340.76	427.2	544.9	701.6	936.8
	SE	17.8	25.7	28.8	66.0	61.4	64.44	69.1	76.1	83.0	130.9
DTX	61	31.9	32.2	77.2	85.8	106.8	121.84	151.8	223.8	384.2	467.0
5 mg/kg	62	61.2	66.3	56.4	27.7	66.3	73.75	64.5	111.4	76.7	65.2
	63	86.0	180.3	264.7	380.7	515.2	540.65	633.9	670.6	664.0	837.8
	64	90.4	113.4	227.3	151.2	356.2	413.10	585.8	714.1	801.1	933.1
	65	141.0	226.8	326.4	386.5	482.8	434.75	475.7	476.2	580.6	576.2
	Mean	82.1	123.8	190.4	206.4	305.5	316.82	382.4	439.2	501.3	575.9
	SE	18.0	35.8	53.0	74.9	93.4	92.30	115.7	119.2	125.8	153.1
DTX	66	29.4	49.6	62.8	138.4	191.8	191.56	353.0	425.9	605.0	646.9
10 mg/kg	67	66.3	71.4	110.5	123.6	186.2	257.03	233.3	322.8	459.5	527.6
	68	70.8	119.1	211.7	234.2	210.8	236.25	305.1	307.2	298.5	358.2
	69	87.1	78.4	153.8	213.2	223.0	214.25	254.8	246.2	373.5	465.5
	70	154.2	323.8	345.7	475.2	556.6	585.00	758.9	647.1	863.3	920.5
	Mean	81.6	128.4	176.9	236.9	273.7	296.82	381.0	389.9	520.0	583.7
	SE	20.5	50.1	48.8	63.2	71.0	72.87	96.7	70.5	99.8	96.3

Table IVb. *Student's t-test (vs. vehicle).*

T-TEST *vs.* Vehicle	Days									
	1	4	8	11	14	17	21	24	28	32
DTX (0.1 mg/lg)	0.995	0.641	0.487	0.243	0.922	0.557	0.992	0.984	0.998	0.775
DTX (0.5 mg/kg)	0.963	0.908	0.439	0.643	0.847	0.693	0.871	0.828	0.871	0.682
DTX (0.75 mg/kg)	0.993	0.778	0.646	0.785	0.807	0.754	0.607	0.794	0.841	0.663
DTX (1 mg/kg)	0.984	0.977	0.399	0.359	0.861	0.523	0.888	0.782	0.838	0.686
DTX (2.5 mg/kg)	0.982	0.539	0.701	0.833	0.636	0.975	0.774	0.765	0.817	0.953
DTX (5 mg/kg)	0.975	0.644	0.967	0.568	0.963	0.873	0.621	0.474	0.355	0.258
DTX (10 mg/kg)	0.995	0.774	0.850	0.779	0.707	0.725	0.587	0.315	0.369	0.226

Table Va. *Relative tumor size (day 1=100), averaged tumor size and standard error (SE) values.*

Dosage	Animal No.	Days									
		1	4	8	11	14	17	21	24	28	32
Vehicle	31	100	101	214	166	213	251	296	376	516	741
	32	100	196	230	318	369	377	444	465	640	878
	33	100	188	234	380	384	327	505	494	583	792
	34	100	225	284	346	437	445	701	1037	1265	1560
	35	100	159	191	328	422	555	730	1032	1285	1350
	Mean	100	174	231	307	365	391	535	681	858	1064
	SE		21	15	37	40	52	81	146	172	164
DTX	36	100	291	601	891	851	1336	1421	2036	1845	2162
0.1 mg/kg	37	100	190	198	350	460	552	671	799	979	1005
	38	100	246	308	631	519	652	810	946	1466	1695
	39	100	179	182	286	257	268	269	339	531	526
	40	100	142	207	261	190	218	288	380	547	600
	Mean	100	210	299	484	455	605	692	900	1073	1198
	SE		26	79	121	116	200	211	307	258	318
DTX	41	100	255	515	715	861	770	1031	1063	1336	1628
0.5 mg/kg	42	100	132	166	142	169	193	301	297	485	664
	43	100	134	222	281	248	282	428	391	562	754
	44	100	215	242	409	475	478	455	468	621	808
	45	100	147	324	357	382	653	855	1072	1801	1164
	Mean	100	176	294	381	427	475	614	658	961	1004
	SE		25	61	95	121	108	140	169	260	178
DTX	46	100	113	286	278	314	344	380	480	647	456
0.75 mg/kg	47	100	219	312	474	538	624	819	981	1238	1745
	48	100	153	199	269	242	220	252	385	447	551
	49	100	177	284	428	450	448	599	1058	1384	1379
	50	100	155	224	281	268	290	378	417	546	612
	Mean	100	163	261	346	362	385	486	664	852	949
	SE		17	21	44	57	70	100	146	191	258
DTX	51	100	224	375	540	513	749	667	911	767	889
1 mg/kg	52	100	179	387	744	840	952	1029	1129	1383	1462
	53	100	181	312	306	332	350	439	419	445	522
	54	100	189	189	357	321	383	474	701	1121	1159
	55	100	150	202	268	263	373	431	535	733	965
	Mean	100	185	293	443	454	561	608	739	890	999
	SE		12	42	89	105	122	114	128	163	155
DTX	56	100	273	305	273	334	568	792	1128	1970	2996
2.5 mg/kg	57	100	114	188	265	334	431	534	778	1113	1422
	58	100	108	236	237	343	382	551	644	798	1003
	59	100	172	216	305	267	386	441	531	516	622
	60	100	132	173	341	361	413	494	618	768	1046
	Mean	100	160	224	284	328	436	562	740	1033	1418
	SE		31	23	18	16	34	60	105	253	414
DTX	61	100	101	242	269	335	383	477	703	1206	1466
5 mg/kg	62	100	108	92	45	108	120	105	182	125	106
	63	100	210	308	443	599	629	737	780	772	974
	64	100	125	251	167	394	457	648	790	886	1032
	65	100	161	232	274	342	308	337	338	412	409
	Mean	100	141	225	240	356	379	461	558	680	798
	SE		20	36	66	78	84	113	125	188	241
DTX	66	100	168	213	470	652	651	1199	1447	2055	2198
10 mg/kg	67	100	101	156	174	263	363	329	456	649	745
	68	100	168	299	331	298	334	431	434	421	506
	69	100	90	176	245	256	246	292	283	429	534
	70	100	210	224	308	361	379	492	420	560	597
	Mean	100	147	214	306	366	394	549	608	823	916
	SE		23	25	49	74	68	166	212	311	323

Table Vb. *Student's t-test (vs. vehicle).*

T-TEST *vs*. Vehicle	Days									
	1	4	8	11	14	17	21	24	28	32
DTX (0.1 mg/lg)	–	0.321	0.416	0.202	0.483	0.331	0.508	0.537	0.506	0.720
DTX (0.5 mg/kg)	–	0.943	0.344	0.492	0.638	0.503	0.639	0.922	0.749	0.808
DTX (0.75 mg/kg)	–	0.707	0.280	0.518	0.970	0.948	0.712	0.937	0.984	0.715
DTX (1 mg/kg)	–	0.670	0.201	0.196	0.454	0.236	0.617	0.772	0.897	0.781
DTX (2.5 mg/kg)	–	0.716	0.815	0.586	0.411	0.486	0.797	0.750	0.582	0.451
DTX (5 mg/kg)	–	0.290	0.888	0.395	0.919	0.910	0.606	0.542	0.505	0.387
DTX (10 mg/kg)	–	0.416	0.576	0.977	0.993	0.967	0.944	0.784	0.924	0.693

Table VIa. *Body weights (g) and standard error (SE) values.*

Dosage	Animal No.	Days										
		1	4	8	11	14	17	21	24	28	32	33
Vehicle	31	23.4	23.3	23.6	24.2	24.7	25.1	25.6	25.4	26.0	26.1	26.4
	32	24.5	24.3	24.0	24.4	24.4	25.4	25.6	25.7	26.5	26.6	27.0
	33	23.6	23.9	23.9	24.0	24.0	24.4	25.9	25.6	26.0	26.0	26.1
	34	23.8	23.7	24.8	25.1	24.5	24.6	25.0	25.0	25.6	25.8	25.7
	35	23.0	23.2	23.6	24.1	24.0	24.3	23.6	23.2	22.5	21.6	22.0
	Mean	23.7	23.7	24.0	24.4	24.3	24.8	25.1	25.0	25.3	25.2	25.4
	SE	0.2	0.2	0.2	0.2	0.1	0.2	0.4	0.5	0.7	0.9	0.9
DTX	36	23.0	23.8	23.8	24.5	23.8	24.6	25.0	25.0	25.5	26.0	25.7
0.1 mg/kg	37	25.7	25.4	26.4	26.5	26.6	26.6	27.0	27.0	27.9	27.6	27.7
	38	26.0	25.8	26.1	27.0	26.2	26.7	27.0	26.8	27.4	27.6	28.1
	39	22.8	23.1	22.8	23.3	23.0	23.5	23.6	23.8	24.6	24.8	25.3
	40	21.4	21.3	21.4	22.1	22.2	22.4	22.4	22.2	22.9	22.6	22.4
	Mean	23.8	23.9	24.1	24.7	24.4	24.8	25.0	25.0	25.7	25.7	25.8
	SE	0.9	0.8	1.0	0.9	0.9	0.8	0.9	0.9	0.9	0.9	1.0
DTX	41	22.7	22.9	23.3	22.9	23.4	23.2	23.2	22.6	23.8	23.8	24.5
0.5 mg/kg	42	24.0	24.2	23.8	24.6	24.8	25.8	25.8	26.2	26.9	26.8	27.0
	43	22.2	22.1	22.5	22.5	22.8	23.4	23.8	23.4	23.9	24.2	24.2
	44	25.6	25.3	25.0	26.1	25.0	26.6	27.0	26.4	26.7	27.4	27.3
	45	24.8	24.3	24.8	25.5	26.0	26.6	27.0	26.4	27.6	27.2	27.7
	Mean	23.9	23.8	23.9	24.3	24.4	25.1	25.4	25.0	25.8	25.9	26.1
	SE	0.6	0.6	0.5	0.7	0.6	0.8	0.8	0.8	0.8	0.8	0.7
DTX	46	23.0	22.6	22.9	23.4	23.2	24.0	24.4	23.6	23.6	23.4	23.3
0.75 mg/kg	47	26.0	26.2	26.1	26.3	27.0	27.6	28.2	27.6	28.5	29.2	29.4
	48	22.4	22.9	23.5	24.3	24.2	24.2	24.2	23.8	25.1	25.4	25.7
	49	23.0	23.0	22.6	23.3	23.2	23.0	23.2	23.4	24.1	24.4	24.5
	50	20.9	21.0	20.4	21.7	21.2	21.6	21.8	21.4	21.9	21.8	22.1
	Mean	23.1	23.1	23.1	23.8	23.8	24.1	24.4	24.0	24.6	24.8	25.0
	SE	0.8	0.8	0.9	0.8	0.9	1.0	1.1	1.0	1.1	1.2	1.3
DTX	51	24.4	23.9	23.3	24.1	23.8	24.8	24.6	24.6	24.6	24.6	24.4
1 mg/kg	52	23.3	23.0	23.4	23.9	23.8	23.8	24.4	24.4	24.6	24.2	24.7
	53	22.0	22.2	22.0	22.4	23.4	23.8	23.8	24.0	24.4	24.4	24.5
	54	23.6	23.6	24.0	24.4	24.8	25.4	25.8	25.2	25.8	25.6	26.2
	55	22.6	23.1	22.9	24.0	24.0	24.6	24.8	24.4	25.1	26.0	25.8
	Mean	23.2	23.2	23.1	24.8	24.0	24.5	24.7	24.5	24.9	25.0	25.1
	SE	0.4	0.3	0.3	1.2	0.2	0.3	0.3	0.2	0.3	0.4	0.4
DTX	56	23.8	24.1	24.1	24.3	25.2	25.0	24.6	25.0	26.6	27.2	27.3
2.5 mg/kg	57	21.1	21.6	21.7	21.9	22.0	21.8	21.8	22.2	23.0	23.2	23.4
	58	21.8	21.4	21.7	22.1	22.8	22.6	22.8	23.0	23.6	23.8	23.8
	59	22.9	23.4	23.2	23.8	23.8	24.2	24.6	25.2	25.4	25.2	25.6
	60	27.0	27.5	27.4	28.5	28.0	28.4	28.8	29.0	30.0	30.2	30.7
	Mean	23.3	23.6	23.6	24.1	24.4	24.4	24.5	24.9	25.7	25.9	26.2
	SE	1.0	1.1	1.1	1.2	1.1	1.1	1.2	1.2	1.2	1.3	1.3
DTX	61	23.2	22.2	22.3	21.7	22.0	22.2	23.4	23.0	23.4	23.4	23.9
5 mg/kg	62	23.4	23.3	23.5	24.3	24.4	24.2	24.8	24.4	25.4	25.4	26.0
	63	27.2	27.2	27.4	27.8	28.0	28.0	29.0	28.6	30.4	29.6	30.0
	64	22.7	23.2	23.4	24.1	24.0	24.4	24.8	24.8	25.6	25.6	26.0
	65	23.0	24.0	24.0	24.8	24.6	24.8	25.4	25.0	25.6	26.2	26.6
	Mean	23.9	24.0	24.1	24.5	24.6	24.7	25.5	25.2	26.1	26.0	26.5
	SE	0.8	0.9	0.9	1.0	1.0	0.9	0.9	0.9	1.2	1.0	1.0
DTX	66	23.2	23.5	23.0	23.9	24.6	24.6	25.0	24.4	25.4	25.2	25.1
10 mg/kg	67	22.6	22.2	22.7	23.4	23.0	23.6	23.6	23.0	23.6	23.8	23.4
	68	26.5	26.0	26.0	26.9	26.6	26.8	27.0	24.6	23.8	23.6	22.8
	69	23.1	23.2	23.3	24.2	24.6	25.0	25.0	25.2	25.4	25.4	25.6
	70	25.2	25.0	25.2	25.8	26.2	26.4	26.4	25.4	26.2	25.6	25.4
	Mean	24.1	24.0	24.0	24.8	25.0	25.3	25.4	24.5	24.9	24.7	24.5
	SE	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.4	0.5	0.4	0.6

Table VIb. *Student's t-test (vs. vehicle).*

T-TEST *vs.* Vehicle	Days										
	1	4	8	11	14	17	21	24	28	32	33.0
DTX (0.1 mg/kg)	0.900	0.818	0.906	0.745	0.965	1.000	0.892	0.985	0.778	0.713	0.774
DTX (0.5 mg/kg)	0.776	0.897	0.851	0.958	0.896	0.659	0.812	0.984	0.681	0.597	0.561
DTX (0.75 mg/kg)	0.508	0.552	0.377	0.492	0.574	0.522	0.514	0.384	0.618	0.812	0.782
DTX (1 mg/kg)	0.348	0.180	0.062	0.173	0.219	0.474	0.407	0.385	0.597	0.798	0.747
DTX (2.5 mg/kg)	0.756	0.945	0.746	0.847	0.971	0.766	0.638	0.939	0.788	0.667	0.664
DTX (5 mg/kg)	0.789	0.741	0.879	0.861	0.782	0.968	0.749	0.866	0.592	0.563	0.447
DTX (10 mg/kg)	0.573	0.682	0.933	0.502	0.333	0.430	0.729	0.483	0.630	0.633	0.379

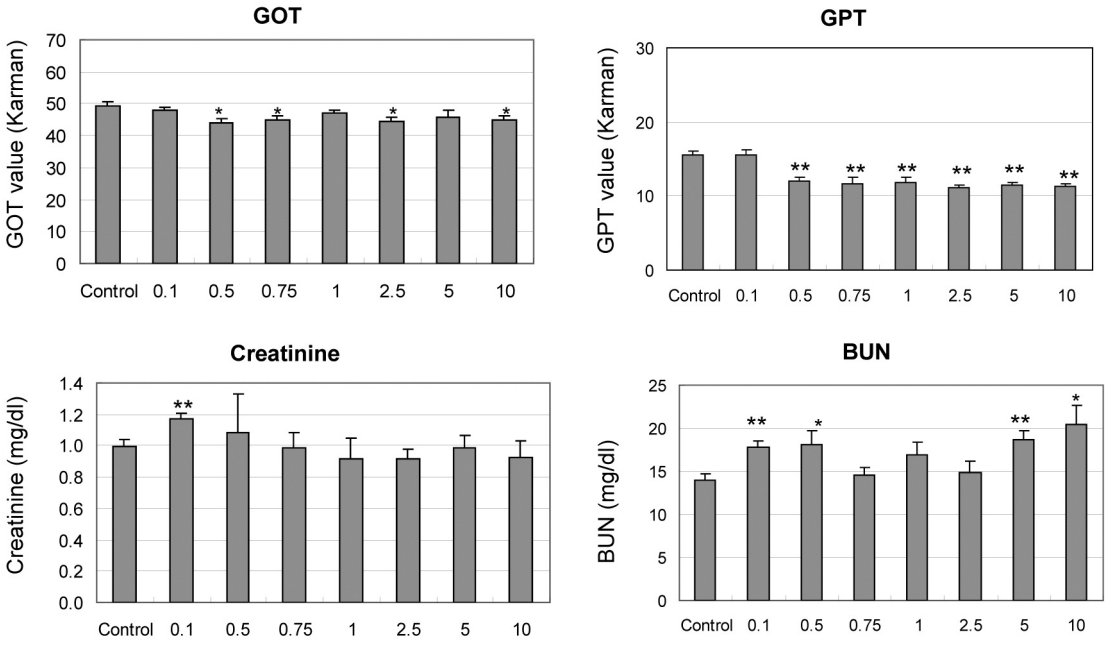

Figure 6. *Average of plasma glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), creatinine and blood urea nitrogen (BUN) values in the blood of xenograft mice. Each value represents the mean±SE of five mice. * and ** indicate p<0.05 and 0.01 compared with control using Student's t-test. Each value was estimated with commercially available kits (see the Materials and Methods section for details).*

with 2.5 mg/kg DTX. In many cases, mice with 2.5 mg/kg DTX developed tumor spheres filled with liquid, such as blood plasma and whole blood (Figure 3D), with tumor volume and body weight being higher. There is no precise explanation for this result except that DTX often causes edematous side-effects in clinical use (10). The body weights of mice at 10 mg/kg DTX were reduced one week after DTX administration, which was maintained until the end. This observation could be due to the toxicities of DTX.

Next, we estimated the inhibitory activities of DTX in xenograft mice developed by implantation of a solid tumor because the targets of DTX are usually solid tumors instead of individual cells (Figure 4). In this study, a wide range of DTX doses of 0.1, 0.5, 0.75, 1, 2.5, 5 and 10 mg/kg was subcutaneously administered once a week for 32 days and tumor volume, as well as body weights, were measured (Figure 4). The inhibitory effects of DTX at 5 and 10 mg/kg were the strongest (Figure 4A and 4B). Interestingly, the suppression effects at 2.5 mg/kg were less than those at 0.1 mg/kg and with vehicle. Edema-like effects were noted in most bodies of mice with 2.5 mg/kg DTX (Figure 4D), with tumors being filled with blood serum and other liquid products (Figure 3D, Figure 5). Again, the only known explanation for these edema-like effects is the edema side-effects observed in clinical patients with the administration of DTX (5, 10). The inhibitory activities in xenograft mice with solid tumors were weaker than for individual DU145 cells in Matrigel™ (Figures 3 and 4). This difference is thought to be due to the difficulty to penetrate the solid DU145 tumor (Figure 2B).

The body weights of mice with the solid tumor model were also suppressed by the administration of 10 mg/kg DTX after 20 days (Figure 4C). However, the weights of the body, liver and kidney on the final day nearly matched the control levels (Figure 5). Hypertrophy of the spleen was frequently noted in all conditions and its dose-dependence was unclear (Figure 5). This could be an artificial result attributed to subcutaneous wounding of nude mice because this phenomenon has not been clinically reported. With respect to hematological parameters, the plasma GOT and GPT values were significantly low, except for with 0.1 mg/kg DTX (Figure 6). These significant decreases did not indicate liver toxicity because the values are usually increased in toxic conditions. Plasma creatinine was normal under all conditions, while its value was only higher at the lowest dose (Figure 6); the plasma BUN values were significantly higher (Figure 6). Although an increase in the BUN is reported in the interview form on DTX (5), we do not have an explanation for this observation. The body weights of mice at a dose of 10 mg/kg were clearly suppressed for both xenograft models (Figure 3C and 4C), with this effect being suspected to be a toxic side-effect of DTX.

In conclusion, we performed a systematic trial on the inhibitory activities of DTX in two DU145 xenograft models

(Figure 2) and demonstrated that (i) DTX was more effective in the xenograft model formed by DU145 cell implantation than the model formed by solid DU145 tumor implantation (Figures 3 and 4); (ii) administration of 2.5 mg/kg DTX was critical because the inhibitory activities of 2.5 mg/kg DTX and lower were weak, while DTX showed effective inhibitory activities at 5 and 10 mg/kg; (iii) edema-like effects were observed for both xenograft models at 2.5 mg/kg DTX with no clear explanation for this irregularity; and (iv) suppression of body weight gain was observed with 10 mg/kg DTX in both xenograft models. DTX is now a standard anti-cancer drug for first-line treatment of hormone-refractory prostate cancer cells. However, its clinical prescription is sometimes limited by edematous side-effects, causing, occasionally, severe edema (5, 11). Based on these results, we believe that the 2.5 mg/kg DTX dose was critical in the xenograft model of DU145 tumor growth for evaluating anti-tumor effects and adverse events regarding combination treatments of new compounds leading, eventually, to a better selection of novel hormone-independent prostate cancer agents.

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