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Toxicity of Novel Solubilizer of Paclitaxel, Aceporol 330, in Beagle Dogs

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Abstract – In order to develop an improved paclitaxel formulation vehicle, a micelle forming solubilizer, Aceporol 330 was synthesized. It was previously reported that Aceporol 330 provided the linearity of paclitaxel plasma pharmacokinetics. In this study, the single dose toxicity test and 2-week repeated dose toxicity test of Aceporol 330 was performed in beagle dogs after intravenous administration. Single dose and 2-week repeated dose toxicity test of Aceporol 330 showed fever/generalized erythema, severe vomiting, and diarrhea in beagle dogs. However, those toxicities were less severe than those of Cremophor EL. Blood chemistry analysis of 2-week repeatedly treated beagle dogs with Aceporol 330 showed significant elevation of total cholesterol (TCHO) and triglyceride (TG) compared to that of control group. Cremophor EL also significantly increased total cholesterol (TCHO) and triglyceride (TG) as much as Aceporol 330. Results from this study indicated that Aceporol 330 was less toxic than Cremophor EL. Based on the pharmacokinetic advantages and the low toxicity of Aceporol 330 in single dose and 2-week repeated dose toxicity test, Aceporol 330 has a potential for use as a safer solubilizer for paclitaxel than Cremophor EL.

Keywords □ Aceporol 330, Cremophor EL, toxicity, beagle dogs.

INTRODUCTION

The current paclitaxel formulation vehicle is composed of 1:1 mixture of Cremophor EL (polyethoxylated castor oil; Fig. 1) and dehydrated ethanol. For administration, paclitaxel formulation in Cremophor EL mixture is diluted with 5~20-fold in normal saline or 5% dextrose solution (Nuijen *et al.*, 2001). However, this vehicle is associated with lots of pharmacological, pharmacokinetical and pharmaceutical problems.

Many studies reported severe toxicity of Cremophor EL such as peripheral neurotoxicity and anaphylactic hypersensitivity reactions (Mielke *et al.*, 2006). It has been suggested that the hypersensitivity reactions due to Cremophor EL are related with histamine release and complement activation (Szebeni, 2005). Ethanol intoxications also have been reported when paclitaxel was administered at high doses during short period (Nuijen *et al.*, 2001). Recently, it was demonstrated that the non-linearity

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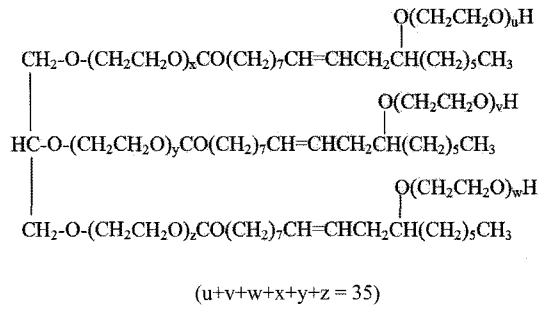
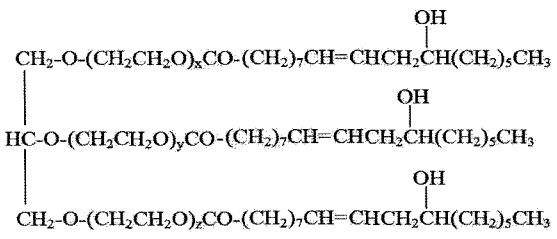
A. Aceporol 330



(u+v+w+x+y+z = 35)

B. Cremophor EL
(major component; polyoxyethyleneglycerol triricinoleate 35)



(x+y+z = 35)

Figure 1. Chemical structure of solubilizer

of paclitaxel plasma pharmacokinetics is also associated with dose- and time- dependent Cremophor EL concentrations (Sparreboom *et al.*, 1999). After dilution in infusion fluids, 0.22 μm in-line filter is required for removal of precipitation of chemicals. Furthermore, Cremophor EL causes the release of the plasticizer diethylhexylphtalate (DEHP) from polyvinylchloride (PVC) infusion sets, necessitating the use of plasticizer-free containers such as glass and polypropylene. Therefore, lots of alternative dosage forms for paclitaxel, including co-solvents, emulsion systems, micro-encapsulation system, cyclodextrins and paclitaxel prodrugs have been evaluated to overcome the pharmaceutical disadvantages of Cremophor EL (Cai *et al.*, 2007; Kang *et al.*, 2004; Le Garrec *et al.*, 2004; Konno *et al.*, 2003).

Several candidate paclitaxel solubilizers were newly synthesized based on the chemical structures of Cremophor EL, in order to improve the pharmacological and pharmacokinetic characteristics of a future paclitaxel solubilizer, as compared to Cremophor EL (Loos *et al.*, 2002; Lee, 2002). One of these candidate vehicles, Aceporol 330 was proved to have an improved loading capacity of paclitaxel as compared to Cremophor EL, and to provide the dose-dependent linearity of paclitaxel concentration in whole blood and plasma of rat (Lee, 2002).

In this study, the single dose toxicity test and 2-week repeated dose toxicity test of a new paclitaxel solubilizer, Aceporol 330 were performed in beagle dogs after intravenous administration and compared its toxicity data with that of Cremophor EL.

MATERIALS AND METHODS

Materials

Aceporol 330 was from Bolak Co. Ltd (Seoul, Korea). Cremophor EL was obtained from BASF Company Ltd. (Seoul, Korea). Ethyl alcohol was purchased from Sigma Chemical Co. (St. Louis, MO, USA). 5% Dextrose solution in glass bottle was from Choongwae Pharma Corporation (Seoul, Korea).

For toxicity test, Aceporol 330 or Cremophor EL was mixed with dehydrated ethyl alcohol (1:1 v/v), and diluted in 5% dextrose in water solution to a final concentration of 5% Aceporol 330/5% dehydrated alcohol or 5% Cremophor EL/5% dehydrated alcohol. Cremophor EL was administered through an in-line filter with a microporous membrane not greater than 0.22 μm.

Animals

Beagle dogs were purchased from Marshall Farm (North Rose, NY, USA). All animals were maintained in a pathogen-free environment air-conditioned at 23±1°C under a 12 h-light/12 h-dark cycle, and allowed free access to food and water until 16-18 h prior to their use in experiments, when they were allowed only water. The animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council).

Single dose toxicity study in beagle dogs

In order to determine the dosage for repeated dose toxicity test in beagle dogs, we performed single dose toxicity test. Six female beagle dogs (5.5 months old) were obtained from Marshall Farm (North Rose, NY, USA). An acclimatization period of 2 weeks was allowed before the start of treatment. Six female beagle dogs weighing 6-7 kg were divided into untreated group, Aceporol 330-treated group and Cremophor EL-treated group. Each group contains two females (6-7 kg). 2 ml/kg Aceporol 330 or 1.5 ml/kg Cremophor EL was infused intravenously into a forelimb vein of beagle dog. Untreated beagle dog group was served as control. The general behavior of the beagle dog was observed during treatment period and any signs of toxicity and lethality were recorded every day. The animals were weighed at day 2, day 6, day 9 and day 14. At the end of the observation period, blood samples were collected from jugular vein of each beagle dog and the animals were sacrificed, dissected, and examined for macroscopically visible changes as described in "Autopsy study". Hematological examinations and biochemical investigations were performed on all beagle dogs as described below. All tissues were weighed and preserved in 10% buffered formaldehyde for histopathological study as described below.

2-Week repeated dose toxicity study in beagle dogs

Beagle dogs (5.5 months old) were obtained from Marshall Farm (North Rose, NY, USA). An acclimatization period of 2 weeks was allowed before the start of treatment. Beagle dogs weighing 6-7 kg were divided into untreated group, Aceporol 330-treated group and Cremophor EL-treated group. Each group contains three female dogs (6-7 kg) and three male dogs (6-7 kg).

Aceporol 330 or Cremophor EL was infused intravenously into a forelimb vein of beagle dog daily for 14 days,

at dose levels of 1 ml/kg/day. Untreated beagle dog group was served as control. The general behavior of the beagle dog was observed during treatment period and any signs of toxicity and lethality were recorded every day. The animals were weighed at day 4, day 7, day 11 and day 14. Water and food consumption of beagle dog determined once a week. Urinalysis was performed for glucose, bilirubin, ketone, specific gravity, blood, protein, pH, urobilinogen, nitrite and leukocytes in urine of all animals using Multistix®10SG (Bayer Co., IN, USA) before treatment and at day 7. The macroscopic and ophthalmoscopic examination was performed for anterior segment of eyes (such as cornea, iris and lens), vitrous body and retina after treatment of 1% atropine sulfate eye drop. At the end of the observation period, blood samples were collected from jugular vein of each beagle dog and the animals were sacrificed, dissected, and examined for macroscopically visible changes as described in "Autopsy study". Hematological examinations and biochemical investigations were performed on all beagle dogs as described below. All tissues were weighed and preserved in 10% buffered formaldehyde for histopathological study as described below.

Hematological investigation and blood chemistry analysis

Hematological examinations were performed using HEMAVET 850 (CDC Technologies, Oxford, CT, USA). Hematology parameters evaluated were white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet count (PLT).

The blood chemistry parameters such as blood urea nitrogen (BUN), glutamyl oxaloacetic transaminase (GOT), glutamyl pyrubic transaminase (GPT), albumin (ALB), glucose (GLU), alkaline phosphatase (ALP), inorganic phosphorus (IP), total bilirubin (TBIL), total cholesterol (TCHO), creatinine (CRE), triglycerides (TG), total protein (TP), calcium, sodium, potassium, chloride ion were determined using FUJI-DRY CHEM slide (Fuji Photo Film Co., Tokyo, Japan) .

Autopsy study

At the end of 2-week repeated dose toxicity study, the animals were sacrificed and dissected. During the process of dissecting, the parenchymatous organs' color, texture, lump and so on were carefully observed. The colour and integrity of the cavities' mucosa were also observed. In beagle dogs, the weight of heart, liver, lung, kidney, brain, spleen, pancrease, bladder, testis, uterine and thymus were measured and recorded.

Histopathological study

Organ pieces (3-5 mm thick) were fixed in 10% buffered formaldehyde for 24 hr and washed in running water for another 24 hr. Samples were dehydrated by passing through 50, 70, 90 and 100% alcohol over a 2 day period, and then embedded in paraffin wax. Paraffin embedded organs were sliced by microtom at the thickness of 4 μm. Routine staining with haematoxylin-eosin and thorough examination using a light microscope were performed.

Statistical analysis

Results were expressed as mean±standard deviation (SD). Statistical significance was determined by a one way analysis of variance (ANOVA) followed by Turkey post hoc comparison for the comparison of the results from the various experimental groups and controls. The differences were considered significant when $P<0.05$.

RESULTS

Single dose toxicity study in beagle dogs

In order to determine the dosage for repeated dose toxicity test in beagle dogs, the single dose toxicity test was performed. All beagle dogs which were treated Cremophor EL or Aceporol 330 showed severe vomiting, fever,

Table I. Body weight changes of beagle dog in single dose toxicity test

	Body weight (kg)				
	Day0	Day2	Day6	Day9	Day14
No treatment	6.50±0.71	6.75±0.35	7.00±0.71	7.25±0.35	7.25±0.35
Aceporol330	6.50±0.71	6.50±0.71	6.75±0.35	6.75±0.35	6.75±0.35
Cremophor EL	6.25±0.35	6.75±0.35	6.50±0.00	6.50±0.00	6.50±0.00

Data represent the mean±SD (n=3; female).

generalized erythema and diarrhea containing mucosa. Vomiting, fever and diarrhea immediately disappeared after stopping the administration of Aceporol 330 or Cremophor EL. After stop of the administration of Aceporol 330 or Cremophor EL, generalized erythema was disappeared gradually within 1 week.

During recovery period, the body weight of beagle dogs in both chemical-treated groups did not show significant changes compared to control group (Table I). Hematological and biochemical changes were not observed after single intravenous administration of Aceporol 330 or Cremophor EL. No pathological changes of the inner organs were discernible with the naked eye during autopsy in all animals tested. 1.5 ml/kg Cremophor EL-treated group showed more severe diarrhea and vomiting than 2 ml/kg Aceporol 330-treated group. Considering the severe diarrhea and vomiting, lower doses of Aceporol 330 and Cremophor EL were needed for 2-week repeated dose toxicity study.

2-Week repeated dose toxicity study in beagle dogs

The animals in all groups survived to the scheduled end of the study. All beagle dogs which were treated Cremophor EL or Aceporol 330 showed severe vomiting and fever/generalized erythema, diarrhea containing mucosa. Vomiting, fever and diarrhea disappeared immediately after stop of administration. After stop of the administration of Aceporol 330 or Cremophor EL, generalized erythema was disappeared gradually within 1 week. Cremophor EL-treated group showed more severe diarrhea and vomiting than Aceporol 330-treated group. Other overt toxic effects were not observed.

Body weight developments of Cremophor EL-treated group or Aceporol 330-treated group were not significantly different from that of controls (Table II). Food consumption in Aceporol 330-treated group was decreased at day 14 for males, and at day 7 for females (Table III). Water consumption of males in Aceporol 330-treated groups was decreased at day 14 (Table IV).

The anterior segments of eyes were normal for all of animals and no abnormality of blood vessels and optic nerve disc and no bleeding in retina were found. Congestion of retina was observed during administration of Aceporol 330 or Cremophor EL. Congestion of retina was

Table II. Body weight changes of beagle dog in 2-week repeated dose toxicity test

	Body weight (kg)				
	Day0	Day2	Day6	Day9	Day14
Male					
No treatment	7.67±1.15	7.67±1.15	7.83±1.04	8.00±0.87	7.83±1.04
Aceporol330	8.67±0.58	8.33±0.58	8.17±0.29	8.33±0.58	8.17±0.29
Cremophor EL	7.67±0.29	8.00±0.50	8.00±0.50	8.50±0.87	8.50±0.87
Female					
No treatment	6.67±0.58	6.67±0.58	6.83±0.76	7.17±0.76	7.17±0.76
Aceporol330	6.50±0.00	6.50±0.00	7.00±0.00	7.00±0.00	7.17±0.29
Cremophor EL	7.00±0.00	7.00±0.00	8.00±0.00	7.83±0.29	7.67±0.29

Data represent the mean±SD (n=3).

Table III. Food consumption of beagle dog in 2-week repeated dose toxicity test

Group	n	Day0	Day7	Day14
	Male			
No treatment	3	280±0	280±0	280±0
Aceporol330	3	280±0	228.3±68.98	120.0±36.06^{*,a}
Cremophor EL	3	280±0	280±0	238.3±42.52
	Female			
No treatment	3	280±0	280±0	280±0
Aceporol330	3	253.3±25.2	193.3±100.5^{*,a}	203.3±55.1
Cremophor EL	3	280±0	320.0±80.5	230.0±62.4

Data represent the mean ± SD (n=3; unit, g).
$^{*}P<0.05$, compared to no treatment control.
$^{a}P<0.05$, compared to Cremophor EL treatment.

Table IV. Water consumption of beagle dog in 2-week repeated dose toxicity test

Group	n	Day0	Day7	Day14
	Male			
No treatment	3	840±196.98	936.7±109.70	910±155.88
Aceporol330	3	823.3±232.45	550.0±213.78	316.7±104.08*,a
Cremophor EL	3	686.7±110.15	740.0±242.49	710.0±175.78
	Female			
No treatment	3	700±100	816.7±76.4	666.7±202.1
Aceporol330	3	753.3±221.9	590.0±300.5	400.0±132.3
Cremophor EL	3	813.3±130.5	816.7±317.5	416.7±301.4

Data represent the mean ± SD (n=3; unit, ml).
*$P<0.05$, compared to no treatment control.
[a]$P<0.05$, compared to Cremophor EL treatment.

Table V. Organ weight of beagle dog after 2-week repeated dose toxicity test

	Brain	Liver	Heart	Kidney1	Kidnye2	Bladder
Male						
No treatment	70.5±8.5	268.3±7.6	74.6±8.9	24.9±3.6	23.4±2.8	6.3±2.1
Ace330	60.1±6.7	289.0±11.5	65.9±3.8	23.9±1.1	23.3±2.5	5.6±2.0
CreEL	67.0±3.5	282.3±32.1	69.4±8.0	27.5±3.3	27.2±4.2	5.4±1.1
Female						
No treatment	62.8±6.1	193.4±24.1	54.1±10.0	17.1±3.2	16.7±1.6	3.4±0.8
Ace330	56.6±8.1	244.0±16.0	51.9±4.1	22.3±3.2	23.2±3.4	6.9±3.2
CreEL	55.5±3.8	225.6±9.2	56.7±2.7	22.3±3.4	24.5±2.5	4.1±1.0
	Testis1	Testis2	Lung	Spleen	Pancrease	
Male						
No treatment	4.8±1.7	4.6±1.3	77.1±6.1	23.7±3.1	75.0±37.9	
Ace330	6.0±0.7	6.0±0.9	66.9±3.1	41.6±10.9	73.7±15.8	
CreEL	4.8±1.3	4.7±1.1	77.3±1.9	28.9±4.4	88.5±13.9	
	Uterine	Thymus	Lung	Spleen	Pancrease	
Female						
No treatment	3.8±0.9	9.8±4.8	67.5±8.2	18.3±3.2	61.2±9.4	
Ace330	3.8±0.8	12.8±1.9	66.3±8.8	35.6±5.9	69.2±12.5	
CreEL	4.7±0.6	10.3±1.5	64.4±4.5	26.1±4.2	69.4±12.4	

Data represent the mean ± SD (n=3; unit, g). *Abbreviation:* Ace330, Aceporol 330; CreEL, Cremophor EL.

Table VI. Hematological test for beagle dog after 2-week repeated dose toxicity test

	WBC	RBC	HB	HCT	MCV	MCH	PLT
Unit	10^3/ul	10^6/ul	g/dl	%	fL(=10^{-15}liter)	pg(=10^{-12}g)	10^3/ul
Male							
No treatment	14.25±7.50	8.51±1.99	20.13±5.40	63.80±17.33	74.40±3.70	23.47±1.11	372.00±66.57
Ace330	12.28±0.28	5.46±0.58	12.00±1.77	41.03±3.37	75.23±3.62	22.10±3.64	248.67±78.59
CreEL	24.28±14.51	9.35±3.15	22.03±6.70	70.47±25.10	75.03±1.50	24.10±6.59	323.00±58.66
Female							
No treatment	9.23±0.87	6.43±0.56	16.17±1.07	44.87±5.51	69.60±3.55	25.17±1.40	341.33±17.04
Ace330	9.69±0.94	5.26±0.31	12.70±0.35	37.23±2.08	70.77±0.55	24.13±0.86	273.00±92.68
CreEL	9.24±1.16	6.97±1.08	15.80±0.28	48.30±8.06	69.25±0.78	22.95±3.18	345.00±25.46

Abbreviation: Ace330, Aceporol 330; CreEL, Cremophor EL; WBC, white blood cell count; RBC, red blood cell count; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; PLT, platelet count.
Data represent the mean ± SD (n=3).

reduced immediately after stop of administration. Urinalysis revealed no findings of statistical and toxicological significance in Aceporol 330-treated groups and in Cremophor EL-treated groups. Organ weight changes and hematology of all animals in Aceporol 330-treated group and Cremophor EL-treated group showed no statistically significant difference from control group at the end of the recovery period (Table V-VI). In blood chemistry anlaysis, total cholesterol (TCHO) and triglyceride (TG) of males and females in Aceporol 330 or Cremophor EL-treated groups were significantly elevated compared to that of control group (Table VII). No histopathological abnormalities were found in organs of all animal groups.

DISCUSSION

Paclitaxel, a water-insoluble anticancer agent, has a non-linear pharmacokinetic profile, due to its formulation vehicle Cremophor EL (van Zuylen *et al.*, 2001b). Novel paclitaxel delivery vehicles ideally should not influence the blood distribution of paclitaxel, while at least equal solubility is required and no life-threatening toxicity should occur (Loos *et al.*, 2002).

Various studies have shown that Cremophor EL alters the pharmacokinetics of many drugs including cyclosporine A, etoposide, doxorubicin and paclitaxel (Glederblom *et al.*, 2001). In contrast to the non-linear pharmacokinetics of paclitaxel in Cremophor EL (van Zuylen *et al.*, 2001b), Aceporol 330 was proved to provide the dose-dependent linearity of paclitaxel concentration in whole blood and plasma of rat (Lee, 2002).

The most well known toxicity of paclitaxel dissolved in Cremophor EL is an acute hypersensitivity reaction, such as dyspnea, flushing, rash, chest pain, tachycardia, hypotension, angio-edema, and generalized urticaria (van Zuylen *et al.*, 2001a; Weiss *et al.*, 1990). According to our experiments, all beagle dogs which were treated Cremophor EL or Aceporol 330 showed severe vomiting and fever/generalized erythema, diarrhea containing mucosa. Cremophor EL-treated group showed more severe diarrhea and vomiting than Aceporol 330-treated group.

Histamine release by Cremophor EL was mainly caused by one of its minor components, oleic acid. The cardiac toxicity, such as asymptomatic rhythm disturbances, might also be caused by Cremophor EL through a mechanism of histamine release (Szebeni, 2005). In spite of premedication of high dose corticosteroids, H_1 and H_2 antagonists, flushing and rashes still occur in 41-44% of all patients and major, potentially life-threatening reactions in 1.5-3% (Gelderblom *et al.*, 2001). Recently, it was shown that Cremophor EL-induced complement activation is an important mechanism of the hypersensitivity reaction of Cremophor EL due to binding of naturally occurring anticholesterol antibodies to the hydroxyl-rich surface of Cremophore EL micelles (van Zuylen *et al.*, 2001b). Aceporol 330 which does not contain oleic acid as a component, also showed hypersensitivity reaction like as Cremophor EL, although underlying mechanism was unknown.

The use of Cremophor EL as a vehicle also appears to alter the biochemical properties of lipoproteins, such as high-density lipoproteins and to shift the electrophoretic and density gradient high density lipoproteins to low density lipoproteins. (Singla *et al.*, 2002). It was also previously reported that high concentrations of Cremophor EL may cause hyperlipidemia, possibly resulting in a change in shape of leukocytes in blood smears (Shimomura *et al.*, 1998). In our 2-week repeated dose toxicity test in beagle dogs, total cholesterol (TCHO) and triglyceride (TG) of males and females in Aceporol 330 or Cremophor EL-treated groups were significantly elevated compared to that of control group (Table VII). The levels of triglyceride in Aceporol 330-treated beagle dogs were even higher than that of Cremophor EL-treated groups. However, whether the observed elevation of total cholesterol (TCHO) and triglyceride (TG) after Cremophor EL and Aceporol 330 increases the risk of vascular accidents is, as yet, unknown.

During the experimental period, a decreasing tendency of food and water consumption was sometimes observed in females and males of both Aceporol 330 and Cremophor EL treated dogs, as compared with the corresponding control dogs. Even though the changes in the water and food consumption were not statistically significant, it might be significant because of the use of only 3 animals per group. There were no significant changes in body weight in these groups. Taking into account these findings, it is possible that these fluctuations in food and water consumption are not considered to be due to the treatment of Aceporol 330 or Cremophor EL.

Slight increases of organ weights were observed in liver, spleen and thymus in Aceporol 330 2-week repeated dose treated dogs as well as in Cremophor EL treated dogs. Likewise, there were no significant histopathological changes observed in these tissues. Neither was associ-

Table VII. Blood chemistry analysis of beagle dog after 2-week repeated dose toxicity test

	BUN	GOT	GPT	ALB	GLU	ALP	IP	TBIL
Unit	mg/dl	U/l	U/l	g/dl	mg/dl	U/l	mg/dl	mg/dl
Male								
No treatment	15.20±5.73	33.33±3.21	25.00±6.24	2.77±0.21	116.33±10.21	294.00± 70.16	4.97±0.40	0.33±0.06
Ace330	11.33±1.32	26.00±6.24	30.00±13.23	2.33±0.06	111.33± 10.69	373.00± 135.34	5.33±0.70	0.37±0.06
CreEL	12.03±1.22	22.67±3.51	18.00± 5.00	2.00±0.36	105.67± 17.21	298.00±71.25	5.13± 0.78	0.30±0.10
Female								
No treatment	17.13±1.79	34.33±7.02	28.67±4.73	3.03±0.12	110.33 ±4.51	321.00 ±71.19	6.73±0.68	0.43±0.12
Ace330	13.93±0.59	21.33±2.52	18.67±2.89	2.27±0.21	86.67 ±10.21	178.00 ±31.10	7.47±0.21	0.33±0.06
CreEL	14.70±0.75	33.00±7.21	22.00±4.00	2.40±0.10	91.33 ±8.08	195.67 ±27.23	7.93±0.55	0.40±0.26

Abbreviation: Ace330, Aceporol 330; CreEL, Cremophor EL; BUN, blood urea nitrogen; GOT, glutamyl oxaloacetic transaminase; GPT, glutamyl pyrubic transaminase; ALB, albumin; GLU, glucose; ALP, alkaline phosphatase; IP, inorganic phosphorus; TBIL, total bilirubin.
Data represent the mean±SD (n=3).

Table VII. Blood chemistry analysis of beagle dog after 2-week repeated dose toxicity test (continued)

	TCHO	CRE	TG	Ca	TP	Na	K	Cl
Unit	mg/dl	mg/dl	mg/dl	mg/dl	g/dl	meq/l	meq/l	meq/l
Male								
No treatment	135.00±30.20	0.67±0.06	37.00±3.61	11.10±0.36	5.20±0.36	140.67±2.52	4.87±0.35	105.67±1.53
Ace330	376.33±84.56*	0.60±0.10	87.33±12.42*,†	11.63±0.12	5.47±0.42	138.33±2.08	4.63±0.38	106.00±5.00
CreEL	327.67± 30.53*	0.53±0.06	41.33±3.06*	10.90±1.05	5.50±0.70	140.67±1.15	7.57±5.58	134.67±32.39
Female								
No treatment	219.67±11.59	0.77±0.06	45.67±6.66	11.17±0.25	5.43±0.21	141.67±2.31	5.10±0.20	108.00±1.00
Ace330	450.00±0.00*	0.50±0.10	85.67±8.50*,a	10.37±0.64	5.63±0.25	137.67±1.15	4.57±0.25	108.00±1.00
CreEL	387.00±79.27*	0.47±0.12	53.00±3.00*	10.77±0.06	5.80±0.26	138.67±1.15	4.47±0.29	111.00±0.00

Abbreviation: Ace330, Aceporol 330; CreEL, Cremophor EL; TCHO, total cholesterol; CRE, creatinine; TG, triglycerides; TP, total protein.
*$P<0.05$, compared to no treatment control $^{a}P<0.05$, compared to Cremophor EL treatment.
Data represent the mean ± SD (n=3).

ated with organ dysfunction or tissue damage, so they were not considered to be adverse.

Slight changes in blood biochemical parameters such as decease in GOT, GPT, and ALP in female and an increase in ALP in male were observed in Aceporol 330 2-week repeated dose treated dogs, although they were not statistically significant. Decreasing tendency of GOT, GPT and ALP were also observed in Cremophor EL treated male and female beagle dogs. Hematological analysis showed slight decrease in RBC, HB, and PLT only in Aceporol 330 treated dogs. However, these changes were within the range of normal variability for the parameters (Jeong *et al.* 2007). Thus, they may not be involved in toxicity of Aceporol 330.

Several groups observed that Cremophor EL caused the neurotoxicity such as axonal swelling, vesicular degeneration and demyelination, and that this toxicity is induced by residual unsaturated fatty acids, possibly due to the appearance of peroxidation products (Gelderblom *et al.*, 2001). In single dose toxicity test and repeated toxicity test in beagle dogs, we couldn't find any evidence of neurotoxicity of Cremophor EL and Aceporol 330 even in the histopathological study.

Based on the pharmacokinetic advantages and the lower toxicity, Aceporol 330 has a potential for use as a safer solubilizer for paclitaxel than Cremophor EL.

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