Observations on the safety of Cremaphor® ELP in rats following intravenous administration.

Shirley A. Aguirre*, Robert H. Denlinger, Walter Collette III and Wenhu Huang

Drug Safety Research and Development, Pfizer Global Research and Development, La Jolla Laboratories, San Diego, CA 92121, USA

Received: August 11, 2013; *Accepted:* August 24, 2013

Technical Note

ABSTRACT

Cremaphor® ELP (CrELP) has been used to emulsify and solubilize water-insoluble substances in the pharmaceutical industry for oral, topical and parenteral preparations, but its safety profile via the intravenous route is yet to be established. In the current report, a wide range of CrELP concentrations associated with different viscosities were formulated and administered intravenously to rats in order to evaluate the acute safety and tolerability. Doses of CrELP were administered once in a fixed volume (5 ml/kg) and concentrations tested in mg/kg included 7.5, 75, 150, 375, 750, 1000, 1250, and 1500, with corresponding viscosites of 1,1, 1.08, 1.6, 3.6, 6, 18, 65 centiPoise (mPa.s), respectively. Mortality was observed within minutes of intravenous dosing with 1250 and 1500 mg/kg. Clinical signs of dyspnea, decreased activity, flat body posture, and rough hair coat were observed in rats given 750 or 1000 mg/kg. Plasma potassium (K^+) levels were increased at 24 hours post dose compared to pre-dose values at all doses tested. Histopathologic evaluations of the heart, kidney and lungs revealed myocardial necrosis and inflammation, renal tubular necrosis and pulmonary histiocytosis with hemorrhage. Collectively, the clinical signs, serum potassium levels and histopathogical findings in rats given 750 and 1000 mg/kg were consistent with compromised tissue perfusion. No adverse findings were observed in rats given 7.5, 75, 150 or 375 mg/kg CrELP and 375 mg/kg was considered the no adverse effect level (NOAEL) in this study.

KEY WORDS: Cremaphor® ELP, excipient, intravenous, rats, safety

INTRODUCTION

The choice of drug delivery system for poorly-water soluble drugs is an essential part of the drug development process (1). One of the most well-known formulation vehicles used for a variety of hydrophobic drugs including photosensitizers, sedatives, anesthetics and anticancer drugs is Cremaphor® EL (CrEL) (1). CrEL is a white to off-white viscous liquid with an approximate molecular weight of ~3 kDa and a specific gravity (25°C/25°C) of 1.05–1.06 and produced by the reaction of castor oil with ethylene oxide at a molar ratio of 1:35 (1). Castor oil is a colorless or pale

* Corresponding author: Shirley A. Aguirre, Drug Safety Research and Development, Pfizer Global Research and Development, La Jolla Laboratories, San Diego, CA 92121, USA, Tel: : (858) 526-4073, Fax: (858) 678-8290, email: shirley.aguirre@pfizer.com

yellow viscous fixed oil obtained from the seeds of *Ricinus communis* consisting mainly of the glycerides of ricinoleic, isoricinoleic, stearic and dihydroxystearic acids. Because CrEL is a heterogeneous non-ionic surfactant, it is usually of highly variable composition, with the major component identified as oxylated triglycerides of ricinoleic acid (i.e., polyoxyethylene glycerol triricinoleate 35 (1). CrEL is not an inert compound and is reported to have a range of biological effects with important clinical implications such as causing severe anaphylactoid hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy (1). Therefore, an understanding of the pharmacologic and biologic effects of this vehicle used in drug formulations is essential for the safe administration of poorly soluble drugs.

Cremophor® ELP (CrELP) is a purified grade of CrEL, a polyoxyl-35 castor oil used as nonionic solubilizer and emulsifier in liquid formulations for oral, topical and parenteral administration (2). It is similar to CrEL but with lower water content, free fatty acid content and differences in viscosity. CrELP has also been used in drug development for multiple therapeutic modalities including cancer and inflammation (3, 4)and likely has the same clinical side effects as CrEL.

In the present study, the safety profile of CrELP was investigated in a one day single dose toleration study in rats using escalated intravenous doses. The objective was to understand the *in vivo* tolerability and safety profile at increasing viscosities and concentration in order to further guide dose selections for more chronic toxicological studies.

EXPERIMENTS AND METHODOLOGY

A one-day single dose intravenous toleration study in rats was undertaken. The aim of the study was to investigate the safety profile of a single intravenous dose of CrELP formulated at increasing viscosities and concentrations.

Animals and Husbandry

Standard procedures and conditions were applied in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures involving laboratory animals were reviewed and approved by the Pfizer Inc. Institutional Animal Care and Use Committee associated with the facility. The one-day single dose intravenous study was conducted using male Wistar Han IGS (CRL:WI [Han]) rats that were supplied by Charles River Laboratories, Inc., (Portage, MI, USA). The rats were six to eight weeks old and weighed 200-250g at the study initiation.

The drug used in the study

CrELP is an extra pure grade of CrEL and was supplied by Sigma-Aldrich (St. Louis, MO, USA). All dose levels (7.5, 75, 150, 375, 750, 1000, 1250 and 1500) measured as mg/kg were formulated using sterile water for injection (WFI), with corres-ponding viscosities of 1.0, 1.0, 1.1, 1.6, 3.6, 6, 18, 65 mPa.s, respectively, and administered to the study animals by intravenous injection.

The design of the study

In this one-day single dose toleration study two male rats per group were intravenously dosed with CrELP at various concentrations of 7.5, 75, 150, 375, 750, 1000, 1250, or 1500 mg/kg formulated in sterile water at an injection volume of 5 ml/kg based on the most recent body weight. Because of the large number of dose groups and resource constraints, only two animals were evaluated per group and a negative control group was not included in the evaluation. The safety profile of the excipient was determined by assessing changes in clinical signs and behavior and pre- and post-test body weights. Laboratory examination of hematology parameters i.e., red blood cells (RBC), hemoglobulin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin

concentration (MCHC), red cell distribution width (RDW), absolute reticulocytes (RETIC), platelets (PLT), mean platelet volume (MPV), white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), basophils (BASO), large unstained cells (LUC) and clinical chemistry parameters i.e., albumin, (Alb), glucose (Glu), potassium (K^+), calcium (Ca^{++}), chloride (Cl^-), albumin/globulin ratio (A/G), globulin (glob), phosphorus (P), sodium (Na^+) and total protein (TP) were performed prior to dosing and 24 hours after dosing. Because this study primarily addressed the safety profile of the excipient as opposed to its acute toxicity profile, other serum parameters such as creatinine, blood urea nitrogen, liver enzymes, creatine kinase and troponin were not evaulated. Necropsy was performed 24 hours after treatment, body weights were recorded, and tissues were collected for histopathologic examinations. Brain, adrenal glands, heart, kidneys, liver and lungs were collected and fixed in 10% formalin. Following fixation, tissues were embedded in paraffin blocks, sectioned (5 μm) onto glass slides, and stained with hematoxylin and eosin (H&E) for microscopic evaluation. Due to limited blood volume sampling, pharmacokinetic parameters were not collected.

The grading scheme for the histopathological changes used in all studies ranged from minimal to severe and was based on the percent of the tissue affected. A grade of minimal corresponded to ≤ 10%, mild corresponded to > 10% to ≤ 25%, moderate corresponded with > 25% to ≤ 50%, and severe corresponded to > 50% of the tissue affected.

RESULTS AND DISCUSSIONS

Tolerability

Treatment-related mortality occurred in rats on Day 1 within five minutes of dosing 1250 mg/kg or 1500 mg/kg CrELP. Changes in blood viscosity have been reported to modulate tissue perfusion (5). It is likely that deaths observed at doses of 1250 and 1500 mg/kg were related to a change in blood viscosity. Intravenous administration of CrELP at a viscosity of ≥18 mPa.s likely increased the local viscosity of the blood, subsequently reducing the blood oxygen tension and decreasing tissue perfusion. This theory of decreased tissue perfusion was substantiated by the clinical signs observed in rats given 750 or 1000 mg/kg, which had signs of a breathing disorder (dyspnea), decreased activity, flat body posture, and rough hair coat. Therefore, CrELP formulations at a viscosity of ≥ 3.6 mPa.s appeared to alter tissue oxygenation/perfusion. No clinical signs were observed in rats given 7.5 to 375 mg/kg CrELP. Thus, CrELP formulated at viscosities between 1.0 to 1.6 mPa.s appeared to be well tolerated. Although the higher doses are estimated to be diluted approximately 13 times due to the rat blood volume (generally accepted as 64 ml/kg), it may be reasonable to speculate that such dilution would not occur instantaneously due to circulatory design.

Body Weights, Hematology and Clinical Chemistry

There were no treatment-related changes in body weights and hematological parameters in rats given ≥ 7.5 mg/kg CrELP (Table 1). The absence of findings in these parameters may have been limited by the short duration of the study.

The only clinical chemistry parameter that changed following the intravenous administration of ≥ 7.5 mg/kg CrELP was the serum K^+ level (Table 2). The average serum K^+ levels in male rats at the predose ranged from 5.8 to 6.2 mmol/l. At 24 hours post treatment, the serum K^+ levels were increased and ranged from 6.6 to 7.8 mmol/l. At all doses of CrELP tested, the serum K^+ level increased above the level of the pre-dose values and above the values for the historical control range (4.3 to 6.4 mmol/l) for male Wistar Han rats. Serum K^+ levels were not available (NA) from animals given 1250 or 1500 mg/kg CrELP. The increased serum K^+

Table 1 Hematology values and tested doses and viscosities of Cremophor® ELP

Cremaphor® ELP(mg/kg)	7.5	75	150	375	750	1000	1250	1500
Viscosity (mPa.s) 37°C	1.0	1.0	1.1	1.6	3.6	6.0	18.0	65
RBC (10e6/µl)								
Pre-dose	7.8	8.3	7.2	7.7	7.7	7.2	7.3	7.5
24 hr Post-dose	6.5	6.4	6.2	6.7	6.4	6.2	NA	NA
HGB (g/dL)								
Pre-dose	14.1	15.1	14.3	14.4	14.6	14.2	14.1	14.2
24 hr Post-dose	11.9	12.0	12.1	12.2	12.2	12.0	NA	NA
HCT (%)								
Pre-dose	42.3	46.0	41.0	41.8	42.5	41.0	41.1	40.9
24 hr Post-dose	37.4	37.0	37.1	38.6	37.3	37.6	NA	NA
MCV (fL))								
Pre-dose	54.0	55.5	56.8	54.1	55.3	57.3	57.4	54.7
24 hr Post-Dose	57.2	58.4	59.9	58.0	58.9	60.8	NA	NA
MCH (pg)								
Pre-dose	18.0	18.2	19.8	18.6	19.0	19.9	19.5	19.0
24 hr Post-dose	18.3	18.9	19.5	18.4	19.2	19.3	NA	NA
MCHC (g/dL)								
Pre-dose	33.3	32.9	34.9	34.4	34.4	34.6	34.1	34.9
24 hr Post-dose	31.9	32.1	32.6	31.7	32.7	31.7	NA	NA
RDW (%)								
Pre-dose	13.0	12.9	12.9	12.5	12.1	12.4	12.4	11.6
24 hr Post-dose	12.6	12.9	13.0	12.4	12.0	12.3	NA	NA
RETIC (10e3/ul)								
Pre-dose	259	390	329	289	231	308	322	234
24 hr Post-dose	297	286	325	291	260	288	NA	NA
PLT (10e3/ul)								
Pre-dose	1127	955	1186	1079	1041	1273	1109	1072
24 hr Post-dose	1155	1097	1154	1135	1013	1226	NA	NA
MPV (fL)								
Pre-dose	10.0	10.6	9.8	10.0	9.9	9.9	9.8	9.9
24 hr Post-dose	9.2	9.8	9.6	9.8	10.3	9.5	NA	NA
WBC (10e3/ul)								
Pre-dose	11.7	16.2	8.0	7.3	10.1	8.2	8.4	8.2
24 hr Post-dose	9.6	7.6	8.1	7.9	9.0	6.3	NA	NA
NEUT (10e3/ul)								
Pre-dose	0.9	1.7	1.5	1.2	1.0	1.0	1.1	0.6
24 hr Post-dose	1.0	0.9	1.0	1.2	1.1	0.9	NA	NA
LYM (10e3/ul)								
Pre-dose	10.1	13.5	6.0	5.7	8.5	4.7	6.8	7.1
24 hr Post-dose	8.0	6.1	6.6	6.2	7.5	4.9	NA	NA
MONO (10e3/ul)								
Pre-dose	0.4	0.6	0.3	0.2	0.3	0.2	0.3	0.3
24 hr Post-dose	0.3	0.4	0.3	0.3	0.3	0.3	NA	NA
EOS (10e3/ul)								
Pre-dose	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
24 hr Post-dose	0.1	0.1	0.1	0.1	0.1	0.1	NA	NA
BASO (10e3/ul)								
Pre-dose	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
24 hr Post-dose	0.1	0.1	0.1	0.0	0.1	0.1	NA	NA
LUC (10e3/ul)								
Pre-dose	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
24 hr Post-dose	0.1	0.1	0.1	0.1	0.0	0.1	NA	NA

levels observed at 750 or 1000 mg/kg could be attributed to tissue necrosis and/or muscle damage, decreased ability of the kidneys to excrete potassium (6). The increased serum K^+ levels correlated with histopathologic findings in the heart and kidneys (Table 2).

Table 2 Serum chemistry values and tested doses and viscosities of Cremophor® ELP

Cremaphor® ELP (mg/kg)	7.5	75	150	375	750	1000	1250	1500
Viscosity (mPa.s) 37°C	1	1	1.1	1.6	3.6	6	18	65
K^+ (mmol/L)								
Pre-dose	5.9	6.2	6.2	5.7	5.5	6	5.9	5.8
24 hr Post-dose	6.6	6.4	7.3	8.1	7.5	7.8	NA	NA
Na^+ (mmol/L)								
Pre-dose	145	146	144	143	144	142	144	143
24 hr Post-dose	149	148	148	146	146	146	NA	NA
Cl^- (mmol/L)								
Pre-dose	100	102	99	102	101	100	101	102
24 hr Post-dose	103	104	104	104	104	105	NA	NA
Ca^{++}(g/dL)								
Pre-dose	11.3	10.5	10.9	10.5	10.5	10.6	10.4	10.7
24 hr Post-Dose	13.4	13	12.9	12.5	12.1	12.7	NA	NA
P (mmol/L)								
Pre-dose	8	6	7.4	6	7.2	6.9	7.2	6.7
24 hr Post-dose	12.3	11.7	12.3	12.5	12.1	12.7	NA	NA
Glu (mg/dL)								
Pre-dose	130	127	118	147	127	156	139	154
24 hr Post-dose	253	219	204	286	271	281	NA	NA
Alb (g/dL)								
Pre-dose	4	4.1	4	3.8	3.8	3.9	3.9	3.8
24 hr Post-dose	4.1	4.1	4	4	3.9	3.9	NA	NA
Glob (g/dL)								
Pre-dose	1.8	2	1.9	1.9	1.9	1.8	1.8	1.8
24 hr Post-dose	2.1	2.3	2.2	2.2	2.2	2.2	NA	NA
A/G								
Pre-dose	2.3	2.1	2.1	2.2	2.1	2.2	2.2	2.2
24 hr Post-dose	2	1.8	2.2	2.2	2.2	2.2	NA	NA
TP (g/dL)								
Pre-dose	5.8	6.1	5.9	5.6	5.7	5.6	5.7	5.5
24 hr Post-dose	6.1	6.4	6.2	6.1	6.1	6	NA	NA

Necropsy Gross Observations

Treatment-related gross observations were not observed in any rats dosed with CrELP.

Histopathologic Findings

Tissues from animals that died within 5 minutes of the administration of 1250 or 1500 mg/kg CrELP were not examined by histopathology. Histopathologic findings were observed in the heart, kidneys, lungs and liver of rats given 750 or 1000 mg/kg CrELP and were absent in animals given ≤ 375 mg/kg CrELP. Serum chemistry parameters that would be indicators of organ toxicity were not included in this evaluation.

Mild multifocal necrosis was observed in the hearts of rats given 1000 mg/kg CrELP (Figure 1). The change was characterized by

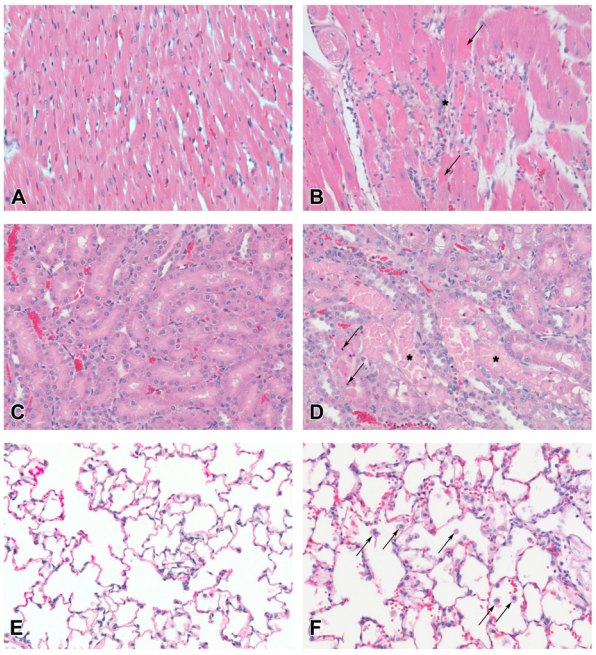

Figure 1 Rat hearts, kidneys and lungs. Normal left ventricle from a rat given 375 mg/kg CrELP shown in (A) at 400x. (B) Myocardial degeneration of the left ventricle from a rat given 1000 mg/kg Cremaphor® ELP. Note the hypereosinophilic myofibers (arrows) and disruption of myofibers with mild mononuclear cell infiltrates (*) at 400x. Normal rat kidney tubules from a rat given 375 mg/kg CrELP shown in (C) at 400x. (D) Kidney from a rat given 1000 mg/kg Cremaphor® ELP. Note the necrotic tubules with pkynotic nuclei (arrows) and luminal eosinophilic casts (*) at 400x. Normal lung from a rat given 150 mg/kg CrELP shown in (E) at 400x. (F) Lung from a rat given 1000 mg/kg Cremaphor® ELP. Note the presence of intra-alveolar histiocytes (arrows) at 400x.

necrosis of individual myocytes surrounded by regenerative myocytes and mononuclear cells, predominantly at the apex of the heart (Figure 1B). Mild multifocal necrosis of kidney tubules at the cortico-medullary junction was observed in the kidneys in rats given 1000 mg/kg CrELP (Figure 1D). The necrosis was characterized by pyknotic cells with granular cytoplasm in scattered individual tubules in the cortico-medullary region of the kidney. Granular and/or cellular casts were found in the lumens of these tubules. At the 750 mg/kg dose level, the changes in the kidneys of the rats were minimal, suggesting a dose related response. In the lungs, a minimal increase in alveolar histiocytes and minimal hemorrhage were observed in rats given 750 or 1000 mg/kg CrELP (Figure 1F). Minimal hemorrhage was also noted in a rat given 375 g/l CrELP (data not shown). There was a minimal diffuse depletion of glycogen was noted in the livers of the rats given 1000 mg/kg CrELP (data not shown). This change was considered an indirect effect of CrELP treatment because of a physiologic response to not eating.

CONCLUSION

This study showed that bolus intravenous administration of CrELP in rats at 1250 or 1500 mg/kg resulted in treatment-related mortality within five minutes of dosing. Intravenous doses of 750 to 1000 mg/kg CrELP treatment at 1000 mg/kg in rats was not well tolerated and produced myocardial necrosis, renal tubular necrosis, pulmonary histiocytosis with hemorrhage and increased serum K^+ levels, all of which are suggestive of tissue hypoperfusion and subsequent cardiovascular collapse. No adverse effects following the intravenous dosing CrELP at 375 mg/kg were observed in the rats. Consequently, this concentration was considered the no observed adverse effect level (NOAEL) in this study. However, the possibility that the NOAEL was between the 375 mg/kg and 750 mg/kg dose levels cannot be excluded. Serum K^+ was increased at all nonlethal doses of CrELP tested. A further study would be required in order to characterize the acute toxicity of CrELP.

Acknowledgements

Special thanks to the following persons for their assistance in preparing this article: Pamela Plumlee for histology assistance, Melissa Marzano for *in vivo* assistance, and Constance Benedict for assistance with the graphics. The work was supported by funding by Pfizer Inc.

REFERENCES

1 Gelderblom H, Verweij J, Nooter KSparreboom A. Cremophor EL., Tthe drawbacks and advantages of

vehicle selection for drug formulation. Eur. J. Cancer, 37: 1590-1598, 2001.

2 BASF "Technical Information. Cremaphor ELP a solubilizer for the pharmaceutical industry," 2002.

3 Richter A, Wiedekind A, Krause M, Kissel T, Haag Rolbrich C., Non-ionic dendritic glycerol-based amphiphiles: Novel excipients for the solubilization of poorly water-soluble anticancer drug Sagopilone. Eur. J. Pharm. Sci., 40: 48-55, 2010.

4 Lee J, Lee Y, Kim J, Yoon MChoi YW. Formulation of microemulsion systems for transdermal delivery of aceclofenac. Arch Pharm Res, 28: 1097-1102, 2005.

5 Lenz C, Rebel A, Waschke KF, Koehler RCFrietsch T., Blood viscosity modulates tissue perfusion: sometimes and somewhere. Transfus Altern. Transfus Med, 9: 265-272, 2008.

6 Stockham SL., Scott MA., eds., Fundamental of veterinary clinical pathology, Iowa State Press Ames, Iowa, 2002.