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Short communication

Disposition of poloxamer 407 in rats following a single intraperitoneal injection assessed using a simplified colorimetric assay

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1. Introduction

Poloxamer 407 (Pluronic® F-127) is a block copolymer comprised of repeating polyoxyethylene and polyoxypropylene units which has found use in controlled drug delivery applications [1–4] and as a barrier for the prevention of post-surgical adhesions in experimental animal models [5,6]. Owing primarily to concerns with potential toxicity caused by poloxamer 407 (P-407) if it were to be used for the prevention of post-surgical adhesions in humans, the time course of P-407 in the plasma of rats following a single intraperitoneal (ip) injection at only one dose level was investigated. Previously, it was reported [7] that ip injection of P-407 caused a dose-dependent hyperlipidemia in rats. Specifically, administration of a 300 mg dose of P-407 to rats resulted in marked hypertriglyceridemia and hypercholesterolemia [7]. It has also been shown previously [8] that the significant increase in triglycerides (TG) following ip injection of P-407 may be mediated by inhibition of capillary-bound lipoprotein lipase (LPL) by P-407. Although it was possible to quantitate the hyperlipidemic response following ip injection of P-407 to rats, the time course of P-407 in the plasma and various organs of the rat could not be determined owing to the lack of a suitable assay. Recently, a spectrophotometric assay was published [9] for the determination of poloxamer 188 (Pluronic® F-68) in an aqueous liver perfusate. Poloxamer 188 is similar in chemical composition to P-407. In this work attempts were made to use the published assay for poloxamer 188 for the detection of P-407 in plasma, urine and the supernatants of kidney and hepatic tissue homogenates following a single 300 mg ip injection of P-407 rats.

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2. Materials and methods

2.1. Materials

P-407 was a gift from the BASF Corp. (Parsippany, NJ) and was used as received. Sterile normal saline was purchased from Baxter Healthcare Corporation (Round Lake, IL). Heparin sodium (5000 U ml^{-1}) was purchased from Elkins Sinn (Cherry Hill, NJ). Cobalt nitrate, ammonium thiocyanate, acetone and ethyl acetate were all obtained from Sigma Chemical Company (St. Louis, MO). Tuberculin syringes used for all injections were 1 ml and were fitted with 1 in, 23 gauge needles purchased from Becton and Dickinson (Rutherford, NJ). Rats (male, Sprague–Dawley, 263–481 g) were purchased from Harlan Laboratories (Indianapolis, IN).

Both an IEC clinical centrifuge and a Fisher Scientific micro-centrifuge (Model 235C) were used for the assay of P-407 in plasma and urine. A Perkin-Elmer Lambda 2 UV/VIS spectrophotometer (Norwalk, CT) was used for the determination of the absorbance values of the P-407–cobalt thiocyanate complexes.

2.2. Colorimetric assay for poloxamer 407

Determination of poloxamer 407 in deionized water (MilliQ®, Millipore, Bedford MA) was based on the following assay. Poloxamer 188 forms a complex with cobalt thiocyanate and the complex in acetone has a maximum absorption at 624 nm [10]. A 3 g amount of cobalt nitrate and 20 g of ammonium thiocyanate was dissolved in and diluted to 100 ml with deionized water (stable for at least 1 month at 25°C) [10]. Volumes of 50 μl of a series of standard P-407 solutions which ranged in concentration from 2 to 20 mg ml^{-1} were introduced into a 1.5 ml polypropylene centrifuge tube to which 0.45 ml of water was added. Six standard concentrations were assayed in triplicate over this concentration range, namely 2, 4, 8, 12, 16 and 20 mg ml^{-1}. Cobalt thiocyanate solution (200 μl) was added to each tube and the solutions were mixed well. Ethyl acetate (0.5 ml) was then added to each tube and the solutions were thoroughly mixed. The mixtures were centrifuged immediately in the micro-centrifuge for 30 s at 10 000*g* and the upper ethyl acetate and aqueous layers aspirated and discarded. The resulting precipitate was then rinsed once with a small volume of water (one drop) and the precipitate transferred into a glass tube. A total of 4 ml of acetone was then added to each tube. The absorbance of the poloxamer 407–cobalt thiocyanate complex in acetone was measured at 624 nm against acetone as a blank [9]. A volume of 50 μl of the above concentrations would yield amounts of P-407 of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg. The standard deviations associated with assaying the amounts of P-407 in triplicate were $\pm 9.4\%$, $\pm 2.7\%$, $\pm 3.4\%$, $\pm 3.2\%$ and $\pm 5.1\%$, respectively.

All rat plasma and supernatants from tissue homogenates which contained P-407 were assayed by the same method as that described above for the aqueous standards subsequent to a standard deproteination procedure. For deproteination of the biological fluids, 0.5 ml of the plasma or tissue supernatant was combined with 2.5 ml of deionized water in a 16 × 100 mm glass culture tube, 1.0 ml of 0.3 N barium hydroxide solution was added and lastly, 1.0 ml of 0.3 N zinc sulfate solution was added to each tube [11]. Addition of 2.5 ml of water diluted the P-407 and decreased the sensitivity of the assay, but was essential to recover quantitatively the P-407 in the sample using this common protein precipitation method. The samples were then centrifuged at 2000*g* for 10 min and the supernatants were collected. To determine the amount of P-407 in each tube, 0.5 ml of each supernatant was then assayed as described above for the P-407 standard solutions prepared with water. Supernatants of liver and kidney tissue homogenates were diluted fourfold during the deproteination procedure. Urine specimens were not deproteinated, but diluted fourfold similar to the supernatants obtained from liver and kidney tissue homogenates. The absorbance of all samples of biological fluids which contained P-407 were determined in a total of 4 ml of acetone. Again, dilution of plasma samples, urine and supernatants of tissue homogenates of ten-, four- and fourfold, respectively, diluted the P-407 and decreased the sensitivity of the assay.

A calibration curve was determined for the detection of P-407 in each of the biological matrices evaluated. As an example, since plasma samples were diluted tenfold during the deproteination procedure, the concentration range of P-407 standards assayed in this matrix was 20–200 mg ml^{-1}. Six concentrations (20, 40, 80, 120, 160 and 200 mg ml^{-1}) were assayed in duplicate. Similarly, the standards used for the urine and supernatants of liver and kidney homogenates used the concentration range 20–200 mg ml^{-1} with the six concentrations noted above assayed in duplicate. The calibration curve based on the absorbance values for the P-407–cobalt thiocyanate complex for the water system yielded a slope of 0.859 ± 0.036 (mean $\pm$ SD) absorbance unit per mgm of P-407. The mean value $\pm$ SD of the slopes obtained for the calibration curves generated in plasma, urine, supernatant of the liver homogenate and supernatant of the kidney homogenate were 0.777 ± 0.027, 0.702 ± 0.034, 0.716 ± 0.021 and 0.832 ± 0.041, respectively. All regression coefficients associated with the calibration curves exceeded 0.9960. The overall mean value $\pm$ SD of the slopes above was 0.777 ± 0.069 with a relative standard deviation of 8.9%. The y-intercepts of the calibration curves for all of the matrices evaluated ranged from 0.020 to 0.032 absorbance units with a mean $\pm$SD of 0.024 ± 0.003. The detection limit for P-407 in water was 0.1 mg when a signal-to-noise ratio of 10 was used as the criterion for a significant absorbance response. However, since all other systems (except urine) were deproteinated and therefore diluted, the detection limits in plasma, liver supernatant and kidney supernatant were 1.0, 0.6 and 0.6 mg, respectively. The detection limit for urine samples which were diluted fourfold, but were not deproteinated, was 0.6 mg. The concentration of P-407 in an individual biological fluid sample was then calculated using the appropriate calibration curve for the particular biological matrix evaluated.

Blank samples of each biological fluid were also analyzed. This spectrophotometric technique for P-407 analysis relies on the measurement of absorbance of a blue P-407–cobalt thiocyanate complex which is precipitated from solution by the addition of ethyl acetate. No blue precipitate was observed with blank samples of any of the biological fluids analyzed in this study.

2.3. Poloxamer 407 solution preparation and administration

The P-407 solution for ip injection was prepared by adding 15 g of P-407 to a tared 100 ml glass beaker followed by the addition of a sufficient volume of normal saline to bring the final weight to 50 g. The beaker was placed on ice overnight to facilitate dissolution of the P-407 using the "cold method" of incorporation [12]. The following day, the polymer solution was gently stirred on ice to ensure a homogeneous solution of P-407 at a concentration of 30% (w/w). A 1 g amount ($\approx$1 ml) of the chilled P-407 solution was drawn up into separate, 1 ml tuberculin syringes. Each of 36 rats (six rats per time point) were then individually administered 300 mg of the P-407 solution by ip injection. All syringes were weighed prior to and following P-407 administration. It should be noted that a different dose of P-407 administered by ip injection may have resulted in a different value of the apparent total body clearance. This study made no attempt to determine pharmacokinetic-related parameters following different doses of P-407. Rather, only a single dose (300 mg) was evaluated to correspond with previous work [8].

2.4. Sample collection

Blood collection was performed at 4, 6, 12, 18, 24 and 48 h following ip injection of P-407 by exposing and then sampling from the descending abdominal aorta of each rat while under ether anesthesia. This procedure for blood sample collection was described previously [8] and typically yields approximately 8 ml of blood per rat. Following collection of blood samples at each time point, the rats were killed by subsequent pneumothorax. Blood samples were collected using 10 ml heparinized syringes and then placed immediately into 10 ml plastic centrifuge tubes. All blood samples collected from the rats at each time point were placed on ice until centrifugation. The

plasma was obtained following centrifugation at 2000*g* and the plasma samples were frozen at −20°C until the time of P-407 assay. The results were expressed as the concentration of P-407 in plasma versus time after injection.

2.5. Urinary excretion study

The rate of urinary excretion of P-407 following a single 300 mg ip injection of the surfactant was determined. Ten rats were each administered a single ip injection of P-407 and then immediately transferred into individual plastic metabolism cages (Biological Resources Laboratory, University of Illinois, Chicago, IL). Food and water were provided ad libitum. Two rats served as sham-injected controls and received 1 ml of normal saline by ip injection. Urine was collected from each rat in 25 ml polypropylene centrifuge tubes (Fisher Scientific; Chicago, IL) over time intervals of 0–24 h, 24–48 h, 48–72 h and 72–96 h. The volume of urine collected over each 24 h urine collection interval was determined using a graduated cylinder. Urine specimens were then frozen at −20°C until the time of P-407 analysis. At the time of analysis, urine samples for each rat were thawed and the prodecure described above for the determination of P-407 in water was followed using the calibration curve for P-407 detection in urine. Results were expressed as the cumulative amount of P-407 detected in the urine versus time post-injection.

2.6. Poloxamer 407 distribution to hepatic and renal tissue

The last experiment was conducted to determine the amount of P-407 found in the supernatants of kidney and liver tissue homogenates following a single 300 mg ip injection of P-407. The time point arbitrarily selected for killing of the rats was 24 h after the ip injection of P-407. Twelve rats were each injected ip with a 300 mg dose of P-407. An additional three rats were also injected ip with normal saline to serve as sham-injected controls. All rats were then ether anesthetized at 24 h following injection of the P-407 and the kidneys and liver excised from each animal. The animal was then immediately killed by pneumothorax. The excised organs were transferred to iced normal saline (4 ml for kidneys; 8 ml for liver) and the organs were then homogenized in a glass/glass homogenizer. The resulting tissue homogenates for each rat were then transferred into separate 25 ml polypropylene centrifuge tubes and centrifuged at 10 000*g* in a Beckman Model J2-21 preparative centrifuge (Palo Alto, CA). The supernatants from each tissue homogenate for each rat were frozen at −20°C until the time of P-407 assay. The determination of the concentration of P-407 in the supernatants was then performed as described above using the appropriate calibration curve.

2.7. Data treatment

The mean plasma P-407 concentration (mg ml^{-1}) ± standard error of the mean (SE) was plotted versus time following the ip injection of P-407. All plasma P-407 concentration–time data were normalized for body weight due to injection of a standard 300 mg dose. The area-under-the-curve (AUC) from 0 to 48 h post-injection was calculated using the trapezoid method [13]. No attempt was made to estimate the AUC from 48 h to time infinity, since an insufficient number of data points during the time period where plasma P-407 concentrations were declining would not permit an accurate estimate of the elimination rate constant. The apparent total body clearance ($CL_{app} = CL_{true}/F$) was calculated by dividing the administered dose (300 mg) by the $AUC_{0\rightarrow 48}$, where F is the bioavailability of P-407 following an ip injection. Since the AUC was truncated at 48 h post-injection, it should be noted that the CL_{app} overestimates the value of CL_{true}. Moreover, since the time course of P-407 in the plasma following a single intravenous dose was not determined in the present study, no estimate of the absolute bioavailability is possible.

Data obtained in urinary excretion studies were treated as follows. The volume of urine excreted over each 24 h urine collection interval was multiplied by the concentration of P-407 determined in the urine sample (mg ml^{-1}). The mean amount ± SD of P-407 excreted in the urine was then calculated for each 24 h urine collection interval.

3. Results

Fig. 1 illustrates the weight-normalized concentration of P-407 in the plasma versus time following a single 300 mg ip injection of P-407 in the rat. The experimental sampling time point at which the maximum plasma P-407 concentration (13.5 mg ml^{-1}) was observed was 12 h post-injection. The value of CL_{app} calculated from the plasma P-407 concentration-time data (cf. Fig. 1) was approximately 2.4×10^{-3} l h^{-1} kg^{-1} or 0.014 ml min^{-1} normalized for a 350 g rat. The mean amount of P-407 excreted in the urine 24 h following an ip injection of P-407 ± SE was 76.3 ± 1.8 mg ($n = 10$). P-407 could not be detected in urine samples obtained from the 24 to 48 h, 48 to 72 h and 72 to 96 h urine collection intervals. At 24 h after ip injection of P-407 to rats, the mean ± SE amounts of P-407 detected in the supernatants of liver and kidney homogenates were 15.9 ± 1.6 mg ($n = 12$) and 3.1 ± 0.26 mg ($n = 12$), respectively.

4. Discussion

The present investigation has characterized the time course of P-407 in plasma following a single 300 mg ip injection to rats. Presumably, the relatively prolonged time period in the blood sampling schedule at which the plasma P-407 concentration was observed to be greatest may be due, in part, to the property of reverse-thermal gelation [12]. The P-407 solution should have formed a semi-solid gel immediately following the ip injection and then slowly dissolved in the peritoneal fluids. Using pulse shearometry, it has been shown previously that the sol-to-gel transition occurred within a few minutes for a 30% (w/w) solution of P-407 at a temperature of only 11°C [14]. The high molecular weight of P-407 may also have contributed to delayed absorption of the molecule. While P-407 is freely water soluble with a hydrophilic to lipophilic balance (HLB) value of 22, the average molecular weight of the copolymer is approximately 12 600. Torres et al. [15] demonstrated using neutral compounds ranging in molecular weight from 18 to 2×10^6 that absorption decreased with increasing molecular weight following ip injection to rats. They demonstrated that only 9.8 and 2.2% of a dose of inulin (MW ≈ 5000) and dextran blue (MW ≈ 2×10^6), respectively, had been absorbed at 1 h following ip injection to rats [15]. Further experimentation is required to elucidate the rate-limiting step for input of P-407 into the systemic circulation following ip injection to rats.

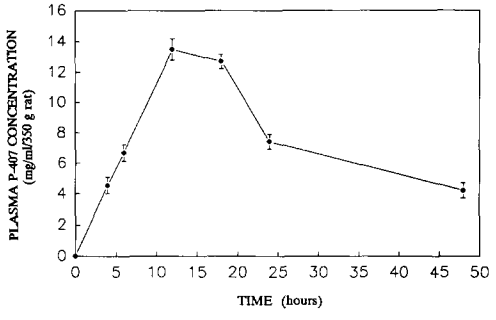

Fig. 1. Concentration of poloxamer 407 (P-407) in the plasma of rats following a single 300 mg intraperitoneal injection. ●, Mean ± SE ($n = 6$).

Previously, it was observed that total cholesterol and TG concentrations in the plasma return to normal values after approximately 96–120 h following a 300 mg ip injection of P-407 to rats (unpublished results). Moreover, an elimination half-life of P-407 ($t_{1/2}$) based on urinary excretion data of 20.9 h ± 0.9 after a standard 300 mg ip injection of P-407 to rats was previously reported [16]. Therefore, about 3% of the dose would remain after five half-lives or approximately 105 h, which is in close agreement with the time required for termination of the hyperlipidemic response. It should be noted that the elimination half-life of P-407 reported earlier (≈21 h) [16] relied on an indirect measurement of P-407 in urine rather that a direct chemical assay. Thus, the previously reported elimination half-life of P-407 in rats should be regarded as only an estimate.

The urinary excretion studies have shown that poloxamer 407 was eliminated in the urine of rats following a single ip injection of the surfactant. Unfortunately, detection of P-407 beyond the first 24 h urine collection interval was not possible with the assay employed. The lack of P-407 detected in later urine samples may have occurred

for several reasons. The ability of the kidneys to filter additional P-407 may have been altered owing to obstructed filtration caused by surfactant which was retained at the glomeruli during the first 24 h urine collection interval. Restriction of hindered filtration of P-407 beginning at approximately 24 h would potentially result in P-407 urine concentrations below the detection limit. It is interesting that Torres et al. [15] demonstrated that less than 1% of a dose of inulin was detected in the urine at 1 h following ip injection to rats. In addition, it was demonstrated previously using rats that an intramuscular injection of inulin formulated in 300 mg of P-407 resulted in a 60% decrease in the apparent plasma clearance (CL/F) of inulin [17]. It was suggested that P-407 altered the rate and extent of glomerular filtration of inulin in a reversible manner [17]. Others [18] have shown that the chemically related poloxamer 188 was distributed to the kidneys following intravenous administration to dogs. However, no decrease in the inulin clearance was observed when poloxamer 188 was simultaneously being cleared by the kidneys [18]. This potentially resulted from the fact that the dose of poloxamer 188 administered in that study using dogs (44 mg kg^{-1}) was almost 23 times less than the dose of poloxamer 407 selected (1 g kg^{-1}) in the present study using rats.

The present study suggests that P-407 may have reversibly altered the filtration capacity of the kidneys in a time-dependent manner. Abe et al. [19] reported severe renal toxicity of P-407 when administered ip to rabbits and mice. The acute ip administered LD_{50} of P-407 in mice was between 1.7 and 5.0 g kg^{-1} body weight [19]. Thus, the surfactant may have caused a reversible alteration in the filtration load for P-407 in the kidneys with increased filtration and/or secretion at earlier time points (0–24 h) and a subsequent return to normal excretion at later times (>24 h). With an average molecular weight of 12 600, negligible glomerular filtration of P-407 would be expected since compounds having a molecular weight greater than 10 000 are generally not filtered to any significant extent [20,21]. P-407-induced renal toxicity with increased excretion of P-407 at earlier times (0–24 h) appears to be a more plausible explanation for the amount of P-407 detected in the 0–24 h urine sample ($\approx$76 mg). The calculated plasma CL_{app} of 0.014 ml min^{-1} for a 350 g rat is well below the glomerular filtration rate (GFR) for a rat of this body weight ($\approx$1.8 ml min^{-1}) [22] assuming that renal excretion is the primary pathway for elimination of P-407 from the body. The assumption that renal excretion is the predominant mechanism for elimination of P-407 is supported by the fact that 75% of the intact, parent molecule was recovered in the urine of dogs 30 h after an intra-arterial injection (100 mg kg^{-1}) of P-407 [23]. Thus, if P-407 was renally elminated similarly to inulin in rats, that is, neither bound to plasma proteins nor reabsorbed in the distal tubule, the plasma CL_{app} of P-407 should approximate the *GFR*. The plasma CL_{app} calculated for P-407 relative to the normal *GFR* (0.014 vs 1.8 ml min^{-1}) suggests that P-407 was cleared extremely slowly owing to either binding with proteins, storage in kidney tissue or extensive reabsorption in the distal tubules. The fact that approximately 25% of the dose of P-407 was excreted in the urine of rats after 24 h supports the contention that the surfactant was somehow able to increase the excretion of P-407 above the rate expected for glomerular filtration alone.

The amount of P-407 which was distributed to the liver 24 h following an ip injection of P-407 to rats in this study was in close agreement with others. Willcox et al. [18] demonstrated that 4.5% of a dose of poloxamer 188 administered intravenously to dogs was recovered in the liver 24 h after administration. It has been shown here using P-407 that 5% (15.9 $\pm$ 1.6 mg) of the administered dose of P-407 was detected in this organ 24 h following an ip injection. The liver contained approximately five times more P-407 on a weight basis than the kidneys when rats were administered 300 mg of the surfactant by ip injection. The preferential uptake of P-407 in hepatic tissue compared with renal tissue explains, in part, several recent findings in our laboratory (unpublished results) concerning alterations in lipid metabolism and lipid concentrations (total cholesterol) following P-407 administration.

In conclusion, the time course of P-407 in the plasma of rats following a single 300 mg ip

injection has been quantitated. Although non-radiolabelled assays for surfactants which contain polyoxyethylene and polyoxypropylene groups have been published previously [10,24], the kinetics associated with the disposition of P-407 in vivo have not been reported owing to the high concentration of the surfactant required for quantitation. The spectrophotometric assay for P-407 used here allowed for the detection of the surfactant in plasma, urine and the supernatants of kidney and liver tissue homogenates. Although the assay is limited in its sensitivity, it does not employ a tritium label as used by others [18]. A tritiated poloxamer molecule has the potential for tritium exchange with body water and fragmentation of the tritium label from the surfactant molecule. Lastly, the results obtained here support more recent findings in this laboratory regarding the surfactant's capacity to induce a reversible hyperlipidemia [7,8].

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