

Bioavailability Following Oral Administration of a Silibinin-Phosphatidylcholine Complex in Cats

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ABSTRACT

The current study determined the intravenous and oral bioavailability of a silibinin-phosphatidylcholine complex (SPC) in cats. Five adult cats were given a single oral dose (10 mg/kg) of SPC and plasma silibinin levels were measured at various time points for 12 hours post-administration. Following a 2-week washout period, a single intravenous dose of SPC (50 mg/cat) was given and plasma silibinin levels were determined at similar time intervals. A pharmacokinetic (PK) comparison of IV silibinin vs oral dosing of SPC in cats showed the following: C_{max} of $87.1\mu\text{M} \pm 44.2$ IV and $5.8\mu\text{M} \pm 3.1$ oral; $\text{AUC}_{0\rightarrow\text{inf}}$ of $86.6\mu\text{M}\cdot\text{hr} \pm 81.4$ IV and $6.1\mu\text{M}\cdot\text{hr} \pm 3.5$ oral; $t_{1/2\lambda}$ of $2.51\text{hr} \pm 1.63$ IV and $3.16\text{hr} \pm 1.74$ oral; V_z/F of $5.65\text{L} \pm 3.66$ IV and $309\text{L} \pm 165$ oral; and CL/F of $1.88\text{L}/\text{hr} \pm 0.99$ IV to $74.7\text{L}/\text{hr} \pm 47.1$ oral. Oral SPC has a bioavailability of approximately 7% in the cat. Although the hepatic levels of silibinin were not determined in this study, the bioavailability of SPC in cats

is an initial step towards determining its effectiveness as a hepatoprotective antioxidant for use in cases of feline liver disease.

INTRODUCTION

Oxidative stress appears to play an important role in the pathogenesis of liver disease in humans, and is likely to serve a similar role in veterinary patients. Approximately one-half of the dogs and cats with chronic liver disease have decreased intra-hepatic levels of reduced glutathione (GSH), a key endogenous antioxidant.¹ It follows that antioxidant supplementation may be an important component of a therapeutic regimen aimed at liver disease in dogs and cats. The milk thistle extract silymarin, composed of multiple flavonolignans including the most active constituent silibinin (synonymous with silybin), is frequently used for its antioxidant properties as a treatment for liver disease.² A poll of chronic liver disease patients at one U S hepatology clinic found 31% of the patients were taking milk thistle.³ In addition to its antioxidant properties, silymarin has been shown to inhibit hepatic stellate cell activation and Kupffer cell function, collagen type I synthesis and fibrin

formation, as well as stimulate hepatocyte regeneration and bile acid production.⁴⁻¹⁰

A number of human clinical trials have now assessed the efficacy of milk thistle (silymarin or silibinin) in the treatment of liver disease, and there is compelling evidence to suggest there are significant therapeutic effects of this compound.¹¹⁻¹³ There is also an abundance of both *in vivo* animal and *in vitro* experimental data showing the hepatoprotective properties of silymarin as an antioxidant and a free radical scavenger.²

One pharmacological result of silymarin administration is an increase in hepatic glutathione content. Specifically, one study found the administration of silymarin to rats elicited a 50% increase in total glutathione content which was maximal by the third day of treatment.¹⁴ Silibinin is a stereoisomer of silymarin with more potent antioxidant properties than either silymarin or crude milk thistle. Complexing silibinin with phosphatidylcholine increases its oral uptake and bioavailability.^{3,15} Administration of a silibinin-phosphatidylcholine complex to rats increased plasma levels, was detected in liver microsomes, and protected against lipid peroxidation.¹⁷ This silibinin-phosphatidylcholine complex has extremely low toxicity in humans.^{3,12,15,16}

Currently, there is no scientific information on the correct dose or evidence of efficacy of milk thistle or the derivatives (silibinin) in the treatment of cats with liver disease. The correct dose of the SPC was recently demonstrated in dogs, but because of the unique characteristics of feline hepatic metabolism, it is difficult to extrapolate between these two species.^{18,19} The purpose of this study was to determine the oral bioavailability of a silibinin-phosphatidylcholine complex (SPC) in cats using standard pharmacokinetic studies.

MATERIALS AND METHODS

Cats

The same five purpose-bred adult cats were used for both the intravenous and oral portions of this study (Cedar River Labo-

ratories, Mason City, IA, USA). All four neutered males and one intact female were unrelated, but had the same birth date and were 13 months old at the time of the study. Their mean body weight was 4.8 ± 0.9 kg (range 3.8 to 5.6 kg). No abnormalities were identified on physical examination, biochemical profile, or complete blood counts. All cats were housed as a group at the Colorado State University (CSU) Veterinary Medical Center for the duration of the study, and all conditions and procedures were in accordance with the CSU Animal Care and Use Committee Guidelines. All cats had *ad libitum* access to dry adult maintenance chow that contained not less than 56.2% protein and 7.8% fat, and not more than 4.8% crude fiber. There are no milk thistle or milk thistle extracts in the diet.

Silibinin-Phosphatidylcholine Complex

Phosphatidylcholine is hygroscopic and stability is dependent on proper handling. Testing of the free powder under conditions of 37°C for 1 month showed no change in silibinin, an 8.5% decrease in phosphatidylcholine content, and a slight increase in water from 2.5 to 3.8%. Under conditions of 25°C over 6 months there were no changes in silibinin, phosphatidylcholine or water content. The capsules were hand filled by using a Fetton Capsule Filler and loader. Each capsule contained approximately 131 mg of SPC material. Capsules were prepared within a 2-hour period at room temperature, over which time the silibinin content was determined to be constant. Each capsule delivered 40 mg of silibinin A+B by analytical analysis.

Sample Acquisition

Prior to both the oral and intravenous portions of the study cats were held off food for 12 hours, and central IV catheters were placed under ketamine sedation, and maintained until after the 12-hour sample acquisition. To determine the pharmacokinetics of orally administered silibinin-phosphatidylcholine complex (SPC) all five cats were administered 10 mg/kg of SPC as a powder combined with cornstarch (31%

silibinin) in a capsule (Nutramax Laboratories, Inc., Edgewood, MD, USA[^]). Capsule administration was followed by 3 ml of water to assure passage through the esophagus. Blood samples were drawn using standard venipuncture technique prior to SPC administration, and then from the central line at the 0.5, 1, 2, 4, 6, 8, and 12 hour time points following SPC administration. Blood samples drawn for silibinin analysis were collected in lithium-heparin tubes, centrifuged, and the plasma stored at -70°C until analyzed.

Following a 14-day washout period, central IV catheters were again placed in the same five cats in a similar manner to facilitate sample acquisition for the intravenous portion of the study. A single IV dose of silibinin (90.5% pure; 50 mg/cat dissolved in absolute ethanol) was administered to each cat via this catheter. Cats appeared sedate for 5-10 minutes following dose administration, likely due to the ethanol content of the formula. Post-treatment samples were drawn at 5 minutes, 15 minutes, and 0.5, 1, 2, 4, 6, 8, and 12 hour time points.

Both sets of samples were prepared, handled, and assayed in the same manner.

Sample Preparation

For the analysis of silibinin in cat plasma a previously developed liquid chromatography-mass spectrophotometry-mass spectrophotometry (LC/MS/MS) based assay was validated.²⁰ A 200 µl aliquot of plasma was transferred to a 1.5 ml polypropylene microcentrifuge tube and 250 ng of naringenin (25 µl of 10 µg/ml) added as an internal standard. Samples were then extracted with 1 ml of acidified ethyl acetate (0.1 % formic acid) for 10 min (vortex). Organic and aqueous layers were separated by centrifugation (10 min, 12000 RCF), and the organic layer removed, evaporated and reconstituted in 1 ml of 20% acetonitrile for LC/MS/MS analysis. Standard curves and QA/QC samples were constructed in plasma by spiking 200 µl of blank plasma with known amounts of silibinin and processing as described above. Analysis of silibinin glucuronate was done essentially as previ-

ously described.²⁰

Mass Spectrometry

Negative ion electrospray ionization (ESI) mass spectra were obtained with a triple quadrupole mass spectrometer with a turbo ionspray source interfaced to a Agilent HPLC system (ABI3200 QTrap, Foster City, CA, USA). Samples were chromatographed with a Waters Phenyl, 2.5µm, 50 X 2 mm column (Waters Corp., Milford, MA, USA). The LC elution was isocratic with 50% acetonitrile containing 10 mM ammonium acetate and 0.1% acetic acid at a flow rate of 400 µl/min and sample injection volume of 20 µl. The analysis time was 5 minutes.

The mass spectrometer settings were: turbo ionspray temperature, 350°C; spray needle voltage, -4500 V; declustering potential (DP), -55 V; entrance potential (EP), -6 V; collision cell entrance potential (CEP), -37 V; collision energy (CE) -37 V; collision cell exit potential (CXP), 0, collision gas, N₂, (CAD) low. Samples were quantified by the internal standard reference method in the MRM mode by monitoring the transition m/z 481 to m/z 125 for the analyte silibinin and m/z 271 to m/z 119 for the internal standard naringenin. The glucuronide conjugates of silibinin were monitored at m/z 657 to 481 respectively and an internal standard for silibinin glucuronate analysis (naringin) was monitored at m/z 579 to m/z 151. Lower and upper limits of detection were 2.5 and 5000 ng/ml. The accuracy of the assay across that range in cat plasma was 92%, and the precision was 5.5%.

Pharmacokinetic Analysis

Analysis of data for the calculation of pharmacokinetic parameters was carried out using noncompartmental analysis with commercial computer software (WinNonlin v.4.1, Pharsight Corp., Mountain View, CA, USA).

RESULTS

A pharmacokinetic (PK) comparison of IV silibinin versus oral dosing of SPC in cats showed the following (Table 1): C_{max} of 87.1µM ± 44.2 IV and 5.8µM ± 3.1 oral;

Table 1. Pharmacokinetic Parameters of Silibinin in Cats Given Either via an IV or Oral Dose.^a

PK Parameter	IV Dose	Oral Dose
C _{max} (μM)	87.1 ± 44.2	5.8 ± 3.1
AUC _{0→inf} (μM•hr)	86.6 ± 81.4	6.1 ± 3.5
t _{1/2λ} (hr)	2.51 ± 1.63	3.16 ± 1.74
V _z /F(L)	5.65 ± 3.66	309 ± 165
CL/F (L/hr)	1.88 ± 0.99	74.7 ± 47.1

a – All pharmacokinetic parameters were calculated by non-compartmental analysis.

AUC_{0→inf} of 86.6μM•hr ± 81.4 IV and 6.1μM•hr ± 3.5 oral; t_{1/2λ} of 2.51hr ± 1.63 IV and 3.16hr ± 1.74 oral; V_z/F of 5.65L ± 3.66 IV and 309L ± 165 oral; CL/F of 1.88L/hr ± 0.99 IV to 74.7L/hr ± 47.1 oral (Figure 1). Based on this data, the oral bioavailability (F) was calculated to be approximately 0.07 (7%).

CONCLUSION

The hepatoprotective properties of silibinin in a number of different species would appear to make this flavonoid a potentially beneficial part of the treatment regimen in a variety of feline liver diseases.²¹⁻²³ For example, silymarin extract protected dogs from amanita toxicity when administered after intoxication.²⁴

One of the limiting factors in the use of polyphenolic flavonoids as antioxidants is their relatively low bioavailability.^{25,26} The ‘phytosome’ complex of silibinin and phosphatidylcholine used in this study is reported to improve bioavailability five-fold in human subjects.^{15,27,28} In the current study, the bioavailability of SPC administered on an empty stomach in a capsule form to cats is between 6-7%. One explanation for the lack of high plasma concentrations and rapid removal from the intravascular space would be efficient hepatic uptake of this compound, which raises the possibility of significant enterohepatic circulation. Plasma silymarin is predominantly cleared through biliary secretion and bile levels greatly exceed serum levels.^{15,29-31} Alternative explanations include ionic trapping, metabolism in other

organs, tissue protein binding, or excretion in urine or bile. Further study is required to determine first-pass hepatic uptake of SPC.

Oral administration of SPC effectively raised plasma levels of silibinin in these study cats. Confirmation of the bioavailability of this complex coupled with absence of side-effects other than temporary sedation most likely due to the ethanol vehicle, suggests that oral administration of SPC is a viable treatment option for felines with clinical disease. A prospective study looking at the effects of SPC administration in cases of spontaneously occurring feline liver disease or other feline diseases where oxidative stress is a likely contributor, such as diabetes mellitus, chronic renal failure, and neoplasia is warranted.³²⁻³⁴

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FOOTNOTES

A Found in Marin[®], Nutramax Laboratories, Inc.

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