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(54) **Titre : UTILISATIONS ET METHODES CONCERNANT LE TRAITEMENT DE MALADIES OU D'AFFECTIONS HEPATIQUES**
(54) **Title: USES AND METHODS FOR THE TREATMENT OF LIVER DISEASES OR CONDITIONS**

(57) **Abrégé/Abstract:**

The present application relates methods and uses of oral diamidines or pharmaceutically acceptable salts thereof for the treatment of liver diseases or conditions.



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USES AND METHODS FOR THE TREATMENT OF LIVER DISEASES OR
CONDITIONS

[1] The present application relates to uses, methods and pharmaceutical composition for the treatment of liver diseases or conditions.

[2] Pentamidine is approved for parenteral administration (intravenous and inhalation) as an anti-protozoal agent. Parenteral (intravenous) pentamidine is also currently being developed for lung cancer, pancreatic cancer and colon cancer.

[3] Based on scientific literature, it is well accepted that pentamidine is not suitable for oral administration due to its poor absorption (Sieve *et al.*: *JOPON*, Vol.11, Vol.2 (April) 1994: pp85-87; Masur H: *N. Eng J. Med.* 327: 1853-1860 (1992); Abdi *et al.* *Handbook of Drugs for tropical parasitic Infections* ISBN0-7484-0168-7; pp117-122).

[4] It is however desirable to give oral forms to patients. Advantages associated with oral forms include higher patient compliance and ease of administration.

[5] In one aspect, there is provided, a method for the targeted treatment of one or more liver conditions comprising the step of orally administering a therapeutically effective amount of at least one diamidine analogue or a pharmaceutically acceptable salt thereof to a human patient in need thereof; wherein the liver condition is liver cancer, liver metastasis, high cholesterol, alcoholic liver disease, cirrhosis, cysts, fatty liver disease (NAFLD), fibrosis, jaundice, primary sclerosing cholangitis (PSC), hemochromatosis, primary biliary cirrhosis or Alpha-1 Antitrypsin Deficiency.

[6] In one aspect, there is provided, the oral use of a therapeutically effective amount of at least one diamidine analogue or a pharmaceutically acceptable salt thereof for the targeted treatment of one or more liver conditions in a human patient in need thereof; wherein the liver condition is liver cancer, liver metastasis, high cholesterol, alcoholic liver disease, cirrhosis, cysts, fatty liver disease (NAFLD), fibrosis, jaundice, primary

sclerosing cholangitis (PSC), hemochromatosis, primary biliary cirrhosis or Alpha-1 Antitrypsin Deficiency.

[7] Figure 1 represents the pharmacokinetics of pentamidine in Mice (IV vs PO).

[8] Figure 2 represents the pentamidine levels following PO administration in liver, kidneys and muscle tissue.

[9] Figure 3 represents the pentamidine mice liver concentrations following different dosage regimen (IV only versus IV followed by PO).

[10] Figure 4 represents the pentamidine mice muscle concentrations following different dosage regimen (IV only versus IV followed by PO).

[11] Figure 5 represents the pentamidine mice kidney concentrations following different dosage regimen (IV only versus IV followed by PO).

[12] Figure 6 represents the pentamidine mice lung concentrations following different dosage regimen (IV only versus IV followed by PO).

[13] Figure 7 represents the pharmacokinetics of pentamidine in dogs depending on the dosage regimen.

[14] Figure 8 represents the pentamidine tissue distribution following different dosage regimen (IV for two days versus PO for three days).

[15] Figure 9. Biodistribution Profiles 24 hours after last dose (Normalized for Equivalent Liver Concentrations of 50 μ M): PENTAMIDINE (pentamidine) PO administration versus IV administration.

[16] Figure 10. Serum ALTs in uninjected control (Con) and PBS alone injected (PBS) mice, and mice GalN/LPS-treated and cotreated with PBS or PENTAMIDINE at 25 and 40 mg/kg (* $P < 0.006$, ** $P < 0.0005$ as compared to PBS + GalN/LPS; $n = 3-7$).

[17] Figure 11. Histology improvement for mice. ALT levels of mice following administration of nothing vehicle (PBS) (A), or GalN/LPS insult to the liver followed by treatment of with vehicle (PBS) (C), 25 or 40 mg/kg (B) (D) of PENTAMIDINE.

[18] Figure 12. Numbers of TUNEL-positive cells are significantly decreased by PENTAMIDINE. Numbers of TUNEL positive cells per high power field (HPF; 200X) in the livers of control and treated mice (*P<0.0002 as compared to PBS + GalN/LPS; n=6-7).

[19] Figure 13. Serum ALTs in mice pretreated with PBS, PENTAMIDINE isethionate 40 mg/kg, or penta-HCL and penta-Tos 20 and 40 mg/kg as indicated, and then administered GalN/LPS (*P<0.008 as compared to PBS + GalN/LPS; n=3-5).

[20] Figure 14. Blood pentamidine PK profile after PO dose of 200 mg/kg in mice for isethionate salt ($\diamond$), HCl salt ($\square$) and tosyate salt (Δ).

[21] **Figure 15.** Body weight (A) and Liver weight (B) for Mice Treated with Vehicle or with PENTAMIDINE. Dotted lines in the figure 15A and 15B represent average values obtained by historical control.

[22] **Figure 16.** Sirius Red Staining of Liver Treated with Vehicle or PENTAMIDINE.

[23] Applicant has found that oral administration of diamidines (e.g., pentamidine) (vs the usual parenteral one) distribute preferentially in the liver.

[24] Applicant has found that oral administration of diamidines (e.g., pentamidine) (vs the usual parenteral one) distribute preferentially in the liver at therapeutic concentrations.

[25] Oral administration refers to administration of the diamidine analogue by mouth. In one aspect the diamidine analogue is swallowed by the patient. Oral administration include the administration of a tablet, a capsule, an elixir, or a solution or other liquid

form of the diamidine analogue by mouth. In one aspect, oral administration also include buccal (dissolved inside the cheek), sublabial (dissolved under the lip) and sublingual administration (dissolved under the tongue).

[26] In one embodiment, the level of diamidines (e.g., pentamidine) is increased in the liver of the patient relative to the diamidines (e.g., pentamidine) level in other tissues and organs.

[27] In one embodiment there is provided, the use of a therapeutically effective amount of at least one diamidine analogue (e.g., pentamidine) or a pharmaceutically acceptable salt thereof in oral form to selectively deliver at least one diamidine analogue (e.g., pentamidine) to the liver of a patient suffering from one or more liver conditions or diseases.

[28] In one embodiment there is provided, a method to selectively deliver at least one diamidine analogue (e.g., pentamidine) to the liver of a patient suffering from one or more liver conditions or diseases comprising orally administering a therapeutically effective amount of at least one diamidine analogue (e.g., pentamidine) or a pharmaceutically acceptable salt thereof.

[29] In one embodiment there is provided, a method to reduced or prevent liver damage comprising orally administering a therapeutically effective amount at least one diamidine analogue (e.g., pentamidine) or a pharmaceutically acceptable salt thereof to a patient in need thereof.

[30] Treatment of Liver Cancer and Liver Metastases

[31] Since diamidines (e.g., pentamidine) preferentially accumulate in the liver in therapeutic concentrations following oral administration, they could be used orally for the treatment liver cancer or liver metastasis.

[32] In one aspect, the liver cancer is intrahepatic bile duct cancer or hepatocarcinoma.

[33] In one aspect, the liver metastasis is liver dominant cancer metastasis or liver limited cancer metastasis.

[34] In oncology, most metastasis occurs in the liver, a vital organ. Rapidly, the primary cancer that has metastasized in the liver becomes life-threatening. For this reason, it is desirable to target the delivery of anti-cancer agents directly to the liver. Because many anti-cancer drugs may have secondary effects, toxicity related to their action on healthy cells elsewhere in the body, it is desirable to have a targeted delivery of anti-cancer agents to the liver. In one embodiment there is provided, the oral use of a therapeutically effective amount of at least one diamidine analogue (e.g. pentamidine) or a pharmaceutically acceptable salt thereof for the targeted treatment liver metastasis, liver dominant cancer metastasis or liver limited cancer metastasis in a patient in need thereof or having received a diagnosis of liver metastasis, liver dominant cancer metastasis or liver limited cancer metastasis.

[35] In one embodiment there is provided, the uses or methods as defined herein, for treating liver dominant colorectal cancer metastasis.

[36] Liver dominant cancer metastasis refers to metastases that are mainly located in the liver (e.g., determination of size, number and type of lesions).

[37] Liver limited cancer metastasis refers to metastases that are only located in the liver (e.g., determination of size, number and type of lesions).

[38] In one aspect, the cancer condition or status of the patient is determined in accordance with the Response Evaluation Criteria in Solid Tumours (RECIST). See for example EUROPEAN JOURNAL OF CANCER 45 (2009) 228–247

[39] In one embodiment there is provided, the uses or methods as defined herein, for treating metastasized cancer.

[40] In one aspect, the patient has one or more of the following conditions:

- a. Inoperable liver tumors, minor lung or bone metastasis or abnormal hepatic enzyme level.

[41] In one embodiment there is provided, the uses or methods as defined herein, wherein the primary cancer originates from squamous cell carcinoma cells, larger cell carcinoma of the lymph node cells, breast cancer cells, colon cancer cells, lung carcinoma cells, melanoma cells, pancreatic cancer cells, leukemia cells, non-small cell lung cancer cells, colon cancer cells, central nervous system (CNS) cancer cells, ovarian cancer cells, renal cancer cells or prostate cancer cells.

[42] In one aspect, the cancer patient is treated as long as the disease is stable or until there is tumor progression (e.g., diseases progression, appearance of new lesions etc.).

[43] In one embodiment there is provided, the use or method of as defined herein wherein the primary cancer originates from pancreatic cancer cells, colon cancer cells, breast cancer cells or ovarian cancer cells.

[44] In one embodiment the diamidine analogues (e.g., pentamidine) are used in combination with standard chemotherapy.

[45] In one embodiment there is provided, an oral pharmaceutical composition comprising at least one diamidine analogue (e.g., pentamidine) or a pharmaceutically acceptable salt thereof and one or more further therapeutic agent indicated for the treatment of cancer.

[46] In one embodiment there is provided, an oral pharmaceutical composition comprising at least one diamidine analogue (e.g., pentamidine) or a pharmaceutically acceptable salt thereof and one or more further therapeutic agent for inhibiting the proliferation of cancer cells or for the treatment of cancer.

[47] Other Liver Conditions or Diseases

[48] Since diamidines (e.g., pentamidine) preferentially accumulate in the liver following oral administration, they could also be used for the treatment of other conditions associated with the liver. In one embodiment, the liver condition is high cholesterol, alcoholic liver disease (including acute alcoholic hepatitis), cirrhosis, cysts, primary biliary cirrhosis, fatty liver disease (NAFLD), fibrosis, jaundice, primary sclerosing cholangitis (PSC), hemochromatosis, primary biliary cirrhosis, or Alpha-1 Antitrypsin Deficiency. See <http://www.rightdiagnosis.com/l/liver/basics.htm> for liver conditions.

[49] In one aspect, liver damage is determined by standard liver function tests and or by imaging (CT, X-Ray, MRI etc.). Liver function tests include bilirubin, ammonia, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), and alkaline phosphatase (ALP).

[50] In one aspect, in the use or method of as defined herein, the patient is not seeking treatment for a viral hepatitis (e.g., A, B, C, D, E or G).

[51] Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)

[52] NAFLD and its more severe form NASH are associated with several diseases (obesity, type 2 diabetes, dyslipidaemia and hypertension), having insulin resistance as the common factor. These conditions cluster to form the insulin resistance or metabolic syndrome, carrying a high risk for cardiovascular complications. NASH itself, as well as pure fatty liver, is an insulin-resistant state, not only in subjects with additional metabolic disorders, but also in lean subjects.

[53] Because the histopathology of NASH resembles that of alcohol-induced steatohepatitis (ASH), these 2 conditions share common pathogenic aspects. Immunological mechanisms play a pivotal role in the pathogenesis of ASH. This has been well demonstrated by studies of patients and experimental animals. In hospitalized patients with severe ASH and NASH, serum levels of several pro-inflammatory

cytokines, including TNF- α , are increased significantly. Cytokine levels correlate well with liver disease severity.

[54] While it is widely acknowledged that TNF- α expression increases in obesity, the mechanisms driving chronic overproduction of TNF- α in obese humans remain obscure. However, the resultant chronic inflammatory state has been implicated in the pathogenesis of the metabolic syndrome that often accompanies obesity. The immunopathogenesis of obesity-related NASH has been studied extensively in the ob/ob mice model. The studies clearly demonstrate that cytokine producing cells in ob/ob livers are Th1 polarized. This microenvironment favours the perpetuation of inflammatory signals. Inhibiting TNF α significantly reduced the hepatic activities of both kinases, thereby supporting the concept that excessive TNF α activity contributes to hepatic insulin resistance in leptin-deficient mice. A strong positive correlation has been noted between hepatic insulin resistance and NASH in many experimental animals and humans.

[55] NAFLD and NASH are initially suspected if blood tests show high levels of liver enzymes. An ultrasound is typically used to confirm the NAFLD diagnosis.

[56] In one aspect, in the uses and methods as described herein the NASH or NAFLD patient will be treated orally with the diamidine analogue in order to prevent, control or reduce liver damage.

[57] In one aspect, in the uses and methods as described herein the patient is a NASH or NAFLD patient that has developed cirrhosis.

[58] In one aspect, in the uses and methods as described herein the NASH or NAFLD patient is overweight or obese, has diabetes, high cholesterol or high triglycerides.

[59] High Cholesterol

[60] High blood cholesterol levels are associated with increased risk of suffering from heart attack and stroke.

[61] In one aspect, the patient is a patient having elevated blood cholesterol levels.

[62] In one aspect, an elevated cholesterol level is a total blood cholesterol level that exceeds 200 mg/dL, that exceeds 220 mg/dL or that exceeds 240 mg/dL.

[63] In one aspect, in the uses and methods as described herein, the patient will be treated orally with the diamidine analogue in order reduce or maintain the total blood cholesterol level below 200 mg/dL, below 220 mg/dL or below 240 mg/dL.

[64] Alcoholic Liver Disease (ALD)

[65] Alcoholic liver disease occurs after years of heavy drinking. Alcohol can cause inflammation in the liver. ALD has three stages: 1) alcoholic fatty liver disease; 2) alcoholic hepatitis and 3) Cirrhosis.

[66] Alcoholic hepatitis (not related to infectious hepatitis) is the second, more serious stage of ALD. It occurs when alcohol misuse over a longer period causes the tissues of the liver to become inflamed.

[67] Damage caused by alcoholic fatty liver disease or Alcoholic hepatitis can usually be reversed if the use of alcohol is stopped.

[68] Cirrhosis is the final stage of alcohol-related liver disease, which occurs when the liver becomes significantly scarred. Cirrhosis is generally not reversible, but stopping drinking alcohol can prevent further damage and significantly increase life expectancy.

[69] In one aspect the ALD is diagnosed with blood test, liver biospsy or imagery (ultrasound scan, computerised tomography (CT) scan).

[70] In one aspect, in the uses and methods as described herein the ALD patient will be treated orally with the diamidine analogue in order to prevent, control or reduce liver damage.

[71] Cirrhosis

[72] Cirrhosis is scarring of the liver caused by many forms of liver diseases and conditions, such as hepatitis and chronic alcohol abuse.

[73] In one aspect the ALD is diagnosed with blood test, liver biospsy or imagery (ultrasound scan, computerised tomography (CT) scan).

[74] In one aspect, in the uses and methods as described herein the cirrhosis patient will be treated orally with the diamidine analogue in order to prevent, control or reduce liver damage.

[75] Cysts

[76] . Cysts are thin-walled structures that contain fluid. Most cysts are single, although some patients may have several. The symptoms associated with liver cysts include upper abdominal fullness, discomfort, or pain.

[77] The cysts are usually found by ultrasound (US) or computed tomography (CT scan).

[78] In one aspect, in the uses and methods as described herein the cysts patient will be treated orally with the diamidine analogue in order to prevent, control or reduce the cysts and/or the symptoms associated with liver cysts.

[79] Fibrosis

[80] Liver fibrosis is the scarring process that represents the liver's response to injury.

[81] Liver fibrosis is usually found by biopsy.

[82] In one aspect, in the uses and methods as described herein the liver fibrosis patient will be treated orally with the diamidine analogue in order to prevent, reduce or control liver fibrosis or inflammation associated/caused by liver fibrosis.

[83] Intra-Hepatic or post-hepatic jaundice

[84] There are three types of jaundice depending on what is causing disruption to the normal removal of bilirubin from the body.

[85] In one aspect, in the uses and methods as described herein the jaundice patient is a patient that suffers from:

- a. intra-hepatic jaundice (also known as hepatocellular jaundice) – the disruption occurs inside the liver. This can be caused by conditions such as Gilbert's syndrome, cirrhosis or other liver damage.
- b. post-hepatic jaundice (also known as obstructive jaundice) – the disruption prevents the bile (and the bilirubin inside it) from draining out of the gallbladder and into the digestive system. This can be caused by conditions such as gallstones or tumours.

[86] In one aspect, in the uses and methods as described herein the intra-hepatic or post-hepatic patient will be treated orally with the diamidine analogue in order to control, reduce or prevent liver damage.

[87] In one aspect, in the uses and methods as described herein the jaundice patient suffers from intra-hepatic jaundice.

[88] Primary sclerosing cholangitis (PSC)

[89] PSC is a disease of the bile ducts. The term "cholangitis" in primary sclerosing cholangitis refers to inflammation of the bile ducts, while the term "sclerosing" describes the hardening and scarring of the bile ducts that result from chronic inflammation.

[90] Primary sclerosing cholangitis is a progressive disease that leads to liver damage and, eventually, liver failure.

[91] In one aspect, in the uses and methods as described herein the PSC patient will be treated orally with the diamidine analogue in order to reduce, control, or prevent liver damage.

[92] Hemochromatosis

[93] Hemochromatosis is an hereditary condition characterised in an excess on iron absorption. The excess iron is stored in organs, especially liver, heart and pancreas. The excess iron can poison these organs, leading to life-threatening conditions such as cancer, heart arrhythmias and cirrhosis.

[94] In one aspect, in the uses and methods as described herein the PSC patient will be treated orally with the diamidine analogue in order to control, prevent or reduce liver damage.

[95] Alpha-1 Antitrypsin Deficiency

[96] The genetic defect in alpha1-antitrypsin (AAT) deficiency alters the configuration of the alpha1-antitrypsin molecule and prevents its release from hepatocytes. As a result, serum levels of alpha1-antitrypsin are decreased, leading to low alveolar concentrations, where the alpha1-antitrypsin molecule normally would serve as protection against antiproteases. The resulting protease excess in alveoli destroys alveolar walls and causes emphysema. The accumulation of excess alpha1-antitrypsin in hepatocytes can also lead to destruction of these cells and ultimately, clinical liver disease.

[97] In one aspect, in the uses and methods as described herein the AAT patient will be treated orally with the diamidine analogue in order to prevent, control or reduce liver damage.

[98] Primary Biliary Cirrhosis (PBC)

[99] PBC is a slow, chronic liver disease which can cause progressive destruction of the bile ducts in the liver. The body attacks the cells lining the bile ducts within the liver as if they are foreign to the body itself. This damage causes poor drainage of bile acids, which leak outwards and damage the normal liver cells. This causes inflammation and scarring which may, after many years become extensive. This widespread damage and scarring is commonly called cirrhosis.

[100] PBC usually diagnosed with blood tests. The presence of AMA (antimitochondrial antibody) is indicative of PBC.

[101] In one aspect, in the uses and methods as described herein the PBC patient will be treated orally with the diamidine analogue in order to prevent, control or reduce liver damage.

[102] In one aspect, in the uses and methods as described herein the PBC patient has tested positive for AMA.

[103] Diamidines Analogues

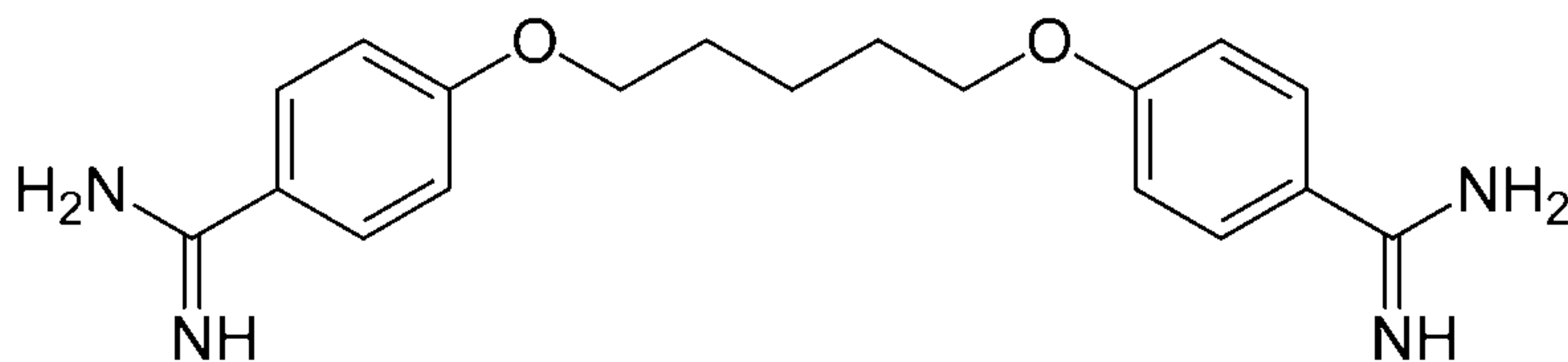
[104] In one aspect, the diamidine analogue is propamidine, butamidine, pentamidine, hexamidine, heptamidine, decamidine and so on, or stilbamidine, furamidine, pafuramidine, or 4,4'(diazamino)dibenzamidine diacetate.

[105] In one aspect, the diamidine analogue is pentamidine.

[106] Pentamidine refers to the free compound or to the compound in salt form, e.g., as the bis(2-hydroxyethanesulfonate) or isethionate salt, HCl, tosylate, mesylate, gluconate or any other pharmaceutically acceptable salt.

[107] In one aspect, pentamidine refers to the free compound or to the compound in salt form, e.g., as the bis(2-hydroxyethanesulfonate) or isethionate salt, mesylate, gluconate or any other pharmaceutically acceptable salt.

[108] Pentamidine is represented by the formula:



[109] In one embodiment, pentamidine is pentamidine bis(2-hydroxyethanesulfonate) or pentamidine isethionate.

[110] Pentamidine is an antiprotozoal drug with additional antiinflammatory activities. It has been reported that pentamidine inhibited the human whole blood production of the chemotactic cytokines (chemokines) interleukin (IL)-8, growth related gene alpha (GRO alpha) and monocyte chemotactic protein-1 (MCP-1). The title compound dose-dependently suppress the lipopolysaccharide (LPS)- and phytohemagglutinin (PHA)-stimulated whole blood generation of these chemokines. The inhibition is specific: when tested at 10 microM, pentamidine has no significant inhibitory effect on the PHA-induced generation of the non-chemotactic cytokines tumor necrosis factor-alpha (TNF-alpha), IL-1 beta, IL-2, IL-4, IL-5, IL-10 and interferon-gamma (IFN-gamma), except for a partial inhibition on IL-6. These findings indicate that pentamidine is a post-transcription acting inhibitor of human chemokine production. This activity may contribute to the anti-inflammatory action ascribed to pentamidine.

[111] **Definitions**

[112] In one aspect, “selective” when referring to the delivery of pentamidine and “targeted” when referring to the treatment using oral pentamidine mean that the amount of pentamidine is increased in the liver of the patient relative to the amount of pentamidine in other tissues and organs (e.g. muscle, lung, kidney and heart) following oral administration if compared to distribution following intravenous

administration. In a further aspect, the amount found in the liver is a therapeutically effective amount.

[113] As used herein the term "patient" means human.

[114] The term "therapeutically acceptable amount" refers to that amount of drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system or animal that is being sought by a researcher or clinician.

[115] The term "treatment" (and corresponding terms "treat" and "treating") includes palliative, restorative, and preventative treatment of a patient.

[116] The term "control liver damage" refers to treatment that eases or reduces the effect or intensity of a condition in a subject without curing the condition.

[117] The term "prevent liver damage" (and the corresponding terms "prevention" and "prophylactic treatment") refers to treatment that prevents the occurrence of a condition in a patient.

[118] The term "reduce liver damage" refers to treatment that halts the progression of or reduces the pathologic manifestations of a condition in a patient.

[119] It is noted in that the present invention is intended to encompass all pharmaceutically acceptable ionized forms (e.g., salts) and solvates (e.g., hydrates) of the compounds, regardless of whether such ionized forms and solvates are specified since it is well known in the art to administer pharmaceutical agents in an ionized or solvated form. It is also noted that unless a particular stereochemistry is specified, recitation of a compound is intended to encompass all possible stereoisomers (e.g., enantiomers or diastereomers depending on the number of chiral centers), independent of whether the compound is present as an individual isomer or a mixture of isomers.

- [120] There is also provided pharmaceutically acceptable salts compounds recited herein. By the term pharmaceutically acceptable salts are meant those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toleune-p-sulphonic, tartaric, acetic, trifluoroacetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Salts derived from amino acids are also included (e.g. L-arginine, L-Lysine). Salts derived from appropriate bases include alkali metals (e.g. sodium, lithium, potassium) and alkaline earth metals (e.g. calcium, magnesium).
- [121] With regards to pharmaceutically acceptable salts, see also the list of FDA approved commercially marketed salts listed in Table I of Berge et al., Pharmaceutical Salts, J. of Phar. Sci., vol. 66, no. 1, January 1977, pp. 1-19.
- [122] It will be appreciated by those skilled in the art compounds can exist in different polymorphic forms. As known in the art, polymorphism is an ability of a compound to crystallize as more than one distinct crystalline or "polymorphic" species. A polymorph is a solid crystalline phase of a compound with at least two different arrangements or polymorphic forms of that compound molecule in the solid state. Polymorphic forms of any given compound are defined by the same chemical formula or composition and are as distinct in chemical structure as crystalline structures of two different chemical compounds.
- [123] It will be appreciated that the amount of compounds required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition for which treatment is required and the age and condition of the patient and will be ultimately at the discretion of the attendant physician.

[124] The desired dose may conveniently be presented in a single dose or as divided dose administered at appropriate intervals, for example as two, three, four or more doses per day. While it is possible that, for use in therapy, the compounds may be administered as the raw chemical it is preferable to present the active ingredient as a pharmaceutical composition. The invention thus further provides a pharmaceutical combination or composition of the compounds as described herein or a pharmaceutically acceptable salt thereof together with one or more pharmaceutically acceptable carriers therefore and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[125] As used herein, the expression "an acceptable carrier" means a vehicle for the combinations and compounds described herein that can be administered to a subject without adverse effects. Suitable carriers known in the art include, but are not limited to, gold particles, sterile water, saline, glucose, dextrose, or buffered solutions. Carriers may include auxiliary agents including, but not limited to, diluents, stabilizers (i.e., sugars and amino acids), preservatives, wetting agents, emulsifying agents, pH buffering agents, viscosity enhancing additives, colors and the like

Pharmaceutical composition of Diamidine Analogues

[126] In one embodiment there is provided, an oral pharmaceutical composition comprising at least one diamidine analogue (e.g., pentamidine) or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable carrier.

[127] Pharmaceutical compositions of pentamidine include those suitable for oral administration. The compositions may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods

well known in the art of pharmacy. All methods include the step of bringing into association the active with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired composition.

[128] Pharmaceutical compositions suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. Pharmaceutical compositions include those suitable for oral administration. The compositions may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired composition.

[129] The diamidines can be conveniently administered in unit dosage form; for example containing 1 to 3000mg, 1 to 2000 mg, 10 to 1500 mg, conveniently 20 to 1000 mg, most conveniently 50 to 700 mg of active ingredient per unit dosage form.

[130] The diamidines can be conveniently administered at least every other day, once, twice or thrice daily. Depending on the treatment selected, the diamidines are administered consecutively for more than 15 days, more than 30 days or more than 45 days.

[131] Typical daily IV doses of pentamidine are 2-8 mg/kg body weight in humans. In one embodiment, the dose of pentamidine can be lower than the typical IV doses. Pentamidine can be conveniently administered in unit dosage form; for example containing 1 to 3000 mg, 1 to 2000 mg, 10 to 1500 mg, conveniently 20 to 1000 mg, most conveniently 50 to 700 mg of active ingredient per unit dosage form.

[132] In one aspect, in the uses and methods described herein, the patient does not suffer from a fungal or protozoa infection (e.g., Leishmania, Pneumocystis jiroveci (carinii) or Trypanosoma infection), viral hepatitis (e.g., hepatitis A, B, B C, D, E or G), immune deficiency (HIV), pneumonia or multiple sclerosis.

Pharmaceutical compositions of further therapeutic agents when used in combination with diamidine (for sequential or simultaneous administration)

[133] One embodiment of the invention includes method and uses thereof comprising a diamidine analogue (e.g., pentamidine) and at least one additional further agent indicated for the treatment of the liver condition.

[134] One embodiment of the invention includes method and uses thereof comprising a diamidine analogue (e.g., pentamidine) in combination with standard therapy indicated for the treatment of the liver condition.

[135] When the liver condition is liver cancer, standard therapy will include standard chemotherapy indicated for the type of cancer (e.g., standard of care).

[136] One embodiment of the invention include method and uses thereof comprising pentamidine and at least one additional further anti-cancer agents for inhibiting the proliferation of cancer cells or for the treatment of cancer in a patient in need thereof.

[137] In one embodiment the further agents include standard chemotherapy. By standard chemotherapy is meant chemotherapy regimen that are used for the treatment of the cancer to be treated.

[138] In one embodiment the further agent include but are not limited to oxaliplatin, cisplatin, mitomycin C, melphalan, carmustine, adriamycin, paclitaxel, docetaxel, 5-fluorouracil, bevacizumab, cetuximab, capecitabine, folinic acid (also known as leucovorin), ionizing irradiation, bleomycin, carboplatin, irinotecan, and/or gemcitabine.

[139] In a further embodiment, the further agent is carboplatin and/or gemcitabine.

[140] In one embodiment the diamidine analogues (e.g., pentamidine) are used in combination with standard therapy indicated for the treatment of the liver condition.

[141] In one embodiment the diamidine analogues (e.g., pentamidine) are not used in combination a cytokine or a granulocyte/macrophage stimulating factor (interferon α , β or γ or IL-2).

[142] When the combination partners employed in the combinations as disclosed herein are applied in the form as marketed as single drugs, their dosage and mode of administration can take place in accordance with the information provided on the package insert of the respective marketed drug in order to result in the beneficial effect described herein, if not mentioned herein otherwise.

[143] One embodiment of the invention includes combination and compositions as described herein wherein the compounds are used sequentially or simultaneously.

- [144] Pharmaceutical compositions include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), transdermal, vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The compositions may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired composition
- [145] The compounds may also be formulated for parenteral administration (e.g., by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.
- [146] For topical administration to the epidermis, the compounds may be formulated as ointments, creams or lotions, or as a transdermal patch. Such transdermal patches may contain penetration enhancers such as linalool, carvacrol, thymol, citral, menthol and t-anethole. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

- [147] Compositions suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.
- [148] Pharmaceutical compositions suitable for rectal administration wherein the carrier is a solid are for example presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.
- [149] Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.
- [150] For intra-nasal administration the compounds or combinations may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs.
- [151] For administration by inhalation the compounds or combinations are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.

[152] Alternatively, for administration by inhalation or insufflation, the compounds or combinations may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or e.g. gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

[153] It will be appreciated that the amount of the further agent required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition for which treatment is required and the age and condition of the patient and will be ultimately at the discretion of the attendant physician. In general however a suitable dose will be in the range of from about 0.1 to about 750 mg/kg of body weight per day, for example, in the range of 0.5 to 60 mg/kg/day, or, for example, in the range of 1 to 20 mg/kg/day. The other agent is conveniently administered in unit dosage form; for example containing 5 to 2000 mg, 10 to 1500 mg, conveniently 20 to 1000 mg, most conveniently 50 to 700 mg of active ingredient per unit dosage form.

[154] The present invention will be more readily understood by referring to the following example. The examples are illustrative of the wide range of applicability of the present invention and are not intended to limit its scope. Modifications and variations can be made therein without departing from the spirit and scope of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the following methods and materials are described. The issued patents, published patent applications, and references that are cited herein are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference. In the case of inconsistencies, the present disclosure will prevail.

EXAMPLES and RESULTS:

[155] Unless indicated otherwise, all experiments are conducted with pentamidine bis(2-hydroxyethanesulfonate)

[156] PO means : by mouth or oral

[157] IV means: intravenous

Example 1: Pharmacokinetics of pentamidine following IV and PO administration

Female Fox Chase SCID CB17 Mice (average animal weight of 0.025kg).

Pharmacokinetic and bioavailability (F): IV administration of 5 mg/kg versus PO administration of 50 mg/kg (pentamidine). Results in mice show a low bioavailability (F) of about 1% (Figure 1).

Oral administration (PO) of 50 mg/kg of pentamidine using DMSO (5%) and sodium caprate (2%) suspension provided significant concentrations of pentamidine in tissues. Liver and kidney levels are significant after PO administration, and are several folds that of blood. Tissues like muscles also contain several folds the concentration found in blood. This result indicates that pentamidine is absorbed significantly in mice when administered orally. (Figure 2)

Example 2: Repeated Oral Administration in Female Fox Chase SCID CB17 Mice (average animal weight of 0.025kg)

In this experiment, mice from all groups were first injected with pentamidine via the IV route on Day 1 (5 mg/kg). Tissue concentrations were assessed 24 hours later (on Day 2), and weekly thereafter (Day 7 and 14) with no subsequent pentamidine administration. Starting on Day 2, groups 2 and 3 were given pentamidine once a day (QD) or twice a day (BID) respectively and tissue levels were measured 24 hours later (Day 3) and weekly thereafter for two weeks.

Results indicate clearly that it is possible to target the liver using the oral administration route. Pentamidine is clearly absorbed and liver concentrations can be maintained to a desired concentration depending on the oral administration regimen (e.g. QD and BID) (Figure 3).

Kidney is targeted by IV administration, and very little by oral administration. Kidney levels are not affected by oral administration, indicating lower systemic exposure. The

same is true for lungs and muscles, which all point to good oral bioavailability and high first pass extraction by liver following oral absorption.

Pentamidine concentrations in other tissues (Kidneys, Lung and muscle) are not affected by the oral administration as much as the liver. This is visible by the similar pentamidine concentration decrease over time following the IV administration in all other tissues. There is no significant difference between groups, with or without PO administration, and with QD or BID PO administration (Figures 4, 5 and 6).

Example 3: A Repeat Dose Oral Capsule Administration or Intravenous Infusion

Pharmacokinetics Study in Male Beagle Dogs. Three oral formulations were tested.

In this example, the pharmacokinetics and biodistribution are studied in Male Beagle Dogs. The Dog is a relevant model since the elimination rate from the different organs, mainly the liver, are closer to that observed in man. Three oral formulations were tested and compared to the injectable formulation:

PO2 capsules: this formulation utilizes Gelucire, Solutol and vitamin E TPGS as absorption enhancers or permeation enhancers. pentamidine/ PEG400/ Gelucire 44/14/ Solutol HS 15/ Vit. E. TPGS/ SLS (19.5%/16.1%/32.2%/24.2%/4.0%/4.0%) dispersion;

PO3 capsules: Spray-Dried powder (70% pentamidine/ 30% PVP K30) with Sodium Caprate and L-Arginine as absorption or permeation enhancers.

PO4 capsules: This formulation does not contain any absorption or permeation enhancer. amorphous dispersion (spray-dried) of pentamidine/Glycerol/SLS (19.5%/76.4%/4.1%).

Pentamidine Spray-Dried formulation for PK study (Group PO2)

Item		Composition	
		%w/w	mg/unit
a)	Spray-Dried Powder (composed of a1 and a2)	75.4	307.0
a1)	<i>Pentamidine bis(2-hydroxyethanesulfonate)</i>	52.8	215.0

a2)	<i>PVP K30</i>	22.6	92.0
b)	Sodium caprate	12.3	50.0
c)	L-Arginine	12.3	50.0
d)	USP water*	15.0	-
e)	Ethanol*	5.0	-
	Fill weight:	100.0	407.0
g)	White Opaque HPMC capsule size "00"	-	120.0
	Total capsule weight:	-	527.0

* Removed during processing, v/w (ml/g of solid)

PEG400 Pentamidine bis(2-hydroxyethanesulfonate) dispersion formulation for PK study (Group PO3)

		Composition	
Item		%w/w	mg/unit
a)	Pentamidine bis(2-hydroxyethanesulfonate)	19.5	215.0
b)	PEG400	16.1	177.4
c)	Gelucire 44/14	32.2	354.5
d)	Solutol HS15	24.2	265.8
e)	Vitamin E TPGS	4.0	43.7
f)	Sodium Lauryl Sulfate	4.0	43.7
	Fill weight:	100.0	1100
g)	White Opaque HPMC capsule size "00"	-	120.0
	Total capsule weight:	-	1220.0

: Glycerol-based amorphous Pentamidine bis(2-hydroxyethanesulfonate) dispersion formulation (Group PO4)

		Composition	
Item		%w/w	mg/unit
a)	Pentamidine bis(2-hydroxyethanesulfonate)	19.5	215.0
b)	Glycerol	76.4	840.0
c)	Sodium Lauryl Sulfate	4.1	45.0
	Fill weight:	100.0	1100
g)	White Opaque HPMC capsule size "00"	-	120.0
	Total capsule weight:	-	1220.0

EXPERIMENTAL DESIGN

The test item was administered twice daily by oral capsule administration for 3 days or once daily by 2-hour peripheral intravenous infusion for 2 days, as shown below:

Treatment Group	Dose Route	Dose Level^e (mg/kg/day)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Infusion Rate (mL/kg/hr)	Number of Males
1.Pent.-OS Formulation IV	IV ^a	4	1	4	2	6 ^c
2.Pent.-OS Formulation PO2	Oral capsules ^b	35 ^e	N/A	N/A	N/A	3 ^d
3. pent.-OS Formulation PO3	Oral capsules ^b	35 ^e	N/A	N/A	N/A	3 ^d
4. pent.-OS Formulation PO4	Oral capsules ^b	35 ^e	N/A	N/A	N/A	3 ^d

^a 2-hour peripheral intravenous infusion once daily for 2 days.

^b Twice daily (12 hours apart) oral capsule administration for 3 days.

^d Necropsy/last sample collection from all animals at the following time-point relative to Day 1: 72 hours post-first dose.

^e All doses reported in pent.-OS salt form equivalent (1.74 mg salt for 1 mg freebase). Capsules are administered at a fixed dose (420 mg/animal/occasion, based on target average weight of animals of 12kg, 35 mg/kg/occasion).

N/A: not applicable

Administration of Dosing Formulations

Group 1 (IV Infusion):

The test item dosing formulation was administered once daily as a 2-hour intravenous infusion via the saphenous and/or cephalic vein using an infusion pump, using a

temporary peripheral vein catheter attached to a syringe, for 2 consecutive days. The dose volume was 4 mL/kg and the infusion rate was 2 mL/kg/hour for all animals. The actual volume administered to each animal was calculated and adjusted based on the most recent practical body weight of each animal. The catheter was flushed with 0.9% Sodium Chloride for Injection, USP (saline) in order to ensure that the whole intended dose was administered to the animals, after which the temporary catheter was removed and discarded.

For dosing accountability purposes, the weight of the infusion syringes during the administration period was recorded prior to the start and following the end of each administration. The animals were restrained on a sling during each 2-hour dosing occasion.

Groups PO2, 3, and 4 (Oral Capsule Administration):

The test item was administered twice daily (targeted time: 12 hours apart) by oral capsule administration for 3 consecutive days to fasted animals (at least 4 hours prior to dosing). Food was returned 2 hours post-dose.

The number of capsules to be administered to each animal was fixed to 2 capsules/animals/occasion. If needed, following dosing, the oral cavity was rinsed with an appropriate volume of reverse osmosis water to facilitate swallowing. The oral cavity was inspected following dosing to ensure that the capsules have been swallowed.

Results

There was no significant difference between the three formulations in terms of pharmacokinetics and bioavailability (F). The average bioavailability was 0.4% when calculated using the AUC of the PO versus IV groups. This low apparent bioavailability hides a significant absorption of the drug, targeted in the liver (Figure 7 and 8).

As can be seen in figure 7 and 8, the oral absorption of pentamidine is predominantly in the liver, and much less in other organs and tissue compared to the IV absorption which

distributes predominantly in the liver, but with much higher concentrations in other organs. In comparison, the oral absorption is clearly targeted to the liver.

The following tables present the different Pharmacokinetic parameters for IV and PO groups, the concentrations of pentamidine in different tissues and the tissue to blood and tissue to liver concentration ratios. These data also demonstrate the targeted delivery to the liver. pentamidine systemic exposure, to the blood compartment and other tissues, is significantly reduced to the profit of the targeted organ.

Table I- Pharmacokinetic Parameters of pentamidine in blood following 2-h IV administration in Beagle Dogs- Day 1

Animal_ID	Cmax (umol/L)	Tmax (hr)	AUClast (hr*umol/L)	Tlast (h)	AUCINF (hr*umol/L)	AUC%Extrap (%)	t _{1/2} (hr)	CL (L/hr/kg)	Vz (L/kg)	MRTlast (hr)
1001	2.16	2	7.35	24	9.09	19.13	NC	1.29	18.59	6.36
1002	1.74	1	10.74	48	20.95	48.74	NC	0.56	22.93	18.84
1003	1.22	1	19.73	72	35.1	43.8	NC	0.34	32.19	30.73
1004	1.04	1	14.33	96	17.89	19.89	35.23	0.66	33.41	38.14
1005	1.43	1	19.11	120	24.54	22.12	47.05	0.48	32.52	45.29
1006	2.2	2	29.35	168	35.82	18.04	NC	0.33	26.52	65.59
Mean	1.632	1.333	16.769	88	23.898	28.619	41.14	0.609	27.694	34.156
SD	0.485	0.516	7.793	51.85	10.317	13.823	21.57	0.359	6.054	20.722
CV%	29.7	38.7	46.5	58.92	43.2	48.3	52.43	59	21.9	60.7

Table II- Pharmacokinetic Parameters of pentamidine in blood following PO administration in Beagle Dogs- Day 1

	Animal_ID	Cmax (umol/L)	Tmax (hr)	AUClast (hr*umol/L)
Group 2	2001	0.04	4	0.26
	2002	0.23	8	1.55
	2003	0.06	8	0.53
	Mean	0.11	6.667	0.778
	SD	0.104	2.309	0.681
	CV%	94.9	34.6	87.5
Group 3	3001	0.09	0.25	0.46
	3002	0.13	1	1.1
	3003	0.06	2	0.41
	Mean	0.093	1.083	0.655
	SD	0.035	0.878	0.39
	CV%	37.6	81	59.5
Group 4	4001	0.06	4	0.62
	4002	0.09	8	0.78
	4003	0.22	3	1.44
	Mean	0.123	5	0.945
	SD	0.085	2.646	0.432
	CV%	69	52.9	45.7

Table III- Pharmacokinetic Parameters of pentamidine in blood following PO administration in Beagle Dogs- Day 3

	Animal_ID	Cmax (umol/L)	Tmax (hr)	AUClast (hr*umol/L)	AUC TAU (hr*umol/L)	Accumulation Index
Group 2	2001	0.09	8	2.2	0.94	NC
	2003	0.2	12	2.91	1.23	NC
	Mean	0.145	10	2.554	1.084	NA
	SD	0.078	2.828	0.496	0.199	NA
	CV%	53.6	28.3	19.4	18.3	NA
Group 3	3001	0.12	12	2.43	1.05	NC
	3002	0.34	12	4.23	1.65	NC
	3003	0.13	2	2.28	1.32	3.9
	Mean	0.197	8.667	2.982	1.342	3.896
	SD	0.124	5.774	1.087	0.303	NA
	CV%	63.2	66.6	36.4	22.6	NA
Group 4	4001	0.21	8	3.85	1.81	16.7
	4002	0.22	1	4.68	2.52	3.87
	4003	0.14	12	2.43	1.11	NC
	Mean	0.19	7	3.655	1.815	10.286
	SD	0.044	5.568	1.138	0.705	9.069
	CV%	22.9	79.5	31.1	38.8	88.2

Table IV- Pharmacokinetic Parameters of pentamidine in tissues- IV dosing Day 1

Organ	Cmax (umol/L)	Tmax (hr)	AUClast (hr*umol/L)
Liver	210	168	27072
Lung	13.4	120	1807.2
Muscle	6.1	168	786
Skin	1.8	168	235.2

Table V- Summary of Tissue-to-Blood Ratios

	Tissue-to-blood ratio							
	Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain	Liver
Group 1	61.57*	26.78*	8.01*	NC	NC	NC	NC	922.38*
	110.56 (45.98)	14.44 (41.19)	48.69 (51.67)	132.92 (27.7)	386.22 (15.15)	58.40 (21.39)	6.19(18.37)	1694.98 (48.41)
Group 2	7.48 (79.19)	14.24 (58.08)	6.68 (58.42)	11.49 (60.99)	39.18 (57.89)	11.59 (70.38)	4.18 (58.65)	3836.52 (64.51)
Group 3	12.40 (103.35)	7.30 (69.26)	7.71 (32.85)	13.16 (60.74)	57.02 (63.30)	13.37 (22.14)	4.06 (29.20)	4195.16 (44.01)
Group 4	16.96 (28.9)	14.73(32.31)	7.65 (21.38)	22 (69.89)	99.4 (62.26)	15.61 (50.76)	4.06 (33.47)	3735.13 (27.87)

Table VI- Summary of Tissue-to-Liver

	Tissue-to-Liver (%)						
	Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain
Group 1	6.67*	0.87*	2.9*				
	6.74 (18.31)	0.91 (25.4)	2.9 (15.11)	9.66 (56.37)	28.4 (51.40)	4.34 (61.27)	0.46 (64.41)
Group 2	0.25 (92.12)	0.41 (67.26)	0.18 (61.23)	0.31 (58.54)	1.13 (66.34)	0.29 (59.17)	0.12 (69.11)
Group 3	0.19 (84.8)	0.11 (57.74)	0.20 (61.78)	0.31 (21.69)	1.26 (27.98)	0.37 (58.33)	0.10 (24.59)
Group 4	0.42 (67.69)	0.51 (72.5)	0.19 (58.8)	0.63 (67.22)	2.86 (61.85)	0.48 (74)	0.12 (55.19)

* Based on AUC

Table VIIa, b and c- Individual Data- Tissue Concentrations and Ratios- Intravenous Dosing

Table VIIa

	Concentrations (μmol/L)								
	Blood	Liver	Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain
1001	0.121	125	9.80	1.63	2.82	10.10	44.80	4.60	0.50
1002	0.25	159	11.80	1.63	5.56	14.20	39.70	7.10	0.80
1003	0.16	208	11.56	1.45	5.28	22.30	53.10	8.50	0.80
1004	0.07	174	11.52	1.23	4.98	13.90	38.30	6.00	0.80
1005	0.08	168	13.39	1.41	5.29	15.80	48.70	8.00	0.70
1006	0.08	210	10.48	1.80	6.11	20.20	55.80	8.20	0.90
Mean	0.13	173.82	11.42	1.52	5.01	16.08	46.73	7.07	0.75
SD	0.07	31.95	1.23	0.20	1.14	4.47	7.08	1.51	0.14
%CV	54.53	18.38	10.78	13.33	22.71	27.78	15.16	21.40	18.38

Table VIIb

Tissue-to-blood ratio							
Liver	Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain
1029	80.95	13.46	23.29	83.47	370.25	38.02	4.13
636	47.20	6.51	22.24	117.36	328.10	58.68	6.61
1297	72.23	9.09	32.98	184.30	438.84	70.25	6.61
2480	164.50	17.52	71.17	114.88	316.53	49.59	6.61
2105	167.42	17.56	66.15	130.58	402.48	66.12	5.79
2622	131.05	22.52	76.33	166.94	461.16	67.77	7.44
1694.99	110.56	14.44	48.69	132.92	386.23	58.40	6.20
820.58	50.84	5.95	25.16	36.92	58.55	12.50	1.14
48.41	45.98	41.19	51.68	27.78	15.16	21.40	18.38

Table VIIc

Tissue-to-Liver (%)						
Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain
7.87	1.31	2.26	8.11	35.97	3.69	0.40
7.42	1.02	3.49	18.44	51.56	9.22	1.04
5.57	0.70	2.54	14.21	33.84	5.42	0.51
6.63	0.71	2.87	4.63	12.76	2.00	0.27
7.95	0.83	3.14	6.20	19.12	3.14	0.27
5.00	0.86	2.91	6.37	17.59	2.58	0.28
6.74	0.91	2.87	9.66	28.47	4.34	0.46
1.23	0.23	0.43	5.45	14.63	2.66	0.30
18.31	25.40	15.11	56.37	51.40	61.27	64.41

Table VIIIa, b-and c Individual Data- Tissue Concentrations and Ratios- Oral Dosing**Table VIIIa**

	24 h post-dose								
	Blood	Liver	Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain
			($\mu\text{mol/L}$)						
2001	0.13	355.21	1.50	1.97	0.79	1.2	5.3	0.9	0.6
2002	NC	496.68	6.05	2.16	0.93	10.6	45.80	6.00	1.20
2003	0.08	395.25	0.27	1.07	0.58	1.1	3.00	1.30	0.30
Mean	0.11	415.71	2.61	1.73	0.77	4.30	18.04	2.73	0.70
SD	0.07	72.92	3.04	0.58	0.17	5.46	24.07	2.84	0.46
%CV	62.45	17.54	116.70	33.65	22.58	126.89	133.45	103.76	65.47
3001	0.11	256.63	0.79	NC	0.64	0.80	2.2	1.6	0.3
3002	0.09	542.31	2.43	0.91	0.95	2.00	8.30	1.40	0.40
3003	0.08	338.15	0.24	0.36	0.54	0.80	4.70	0.80	0.40
Mean	0.09	379.03	1.15	0.63	0.71	1.20	5.07	1.27	0.37
SD	0.02	147.16	1.14	0.46	0.22	0.69	3.06	0.42	0.06
%CV	16.37	38.83	98.72	72.08	30.40	57.74	60.38	32.87	15.75
4001	0.18	484.60	2.77	3.58	1.04	3.00	15.2	4.2	0.9
4002	0.15	560.75	3.37	1.58	1.34	5.90	25.10	2.40	0.70
4003	0.08	381.99	1.04	1.10	0.66	0.80	3.70	0.60	0.20
Mean	0.14	475.78	2.39	2.09	1.01	3.23	14.68	2.40	0.60
SD	0.05	89.71	1.21	1.32	0.34	2.56	10.71	1.80	0.36
%CV	37.55	18.85	50.45	63.05	33.70	79.11	72.98	75.00	60.09

Table VIIIb

Tissue-to-blood ratio							
Liver	Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain
2732.39	11.53	15.15	6.08	9.23	40.87	6.92	4.62
NC	NC	NC	NC	NC	NC	NC	NC
4940.66	3.43	13.33	7.27	13.75	37.50	16.25	3.75
3836.52	7.48	14.24	6.68	11.49	39.18	11.59	4.18
2474.96	5.92	8.27	3.90	7.01	22.69	8.15	2.45
64.51	79.19	58.08	58.42	60.99	57.89	70.38	58.65
2332.96	7.16	NC	5.81	7.27	20.10	14.55	2.73
6025.63	27.01	10.10	10.58	22.22	92.22	15.56	4.44
4226.89	3.03	4.51	6.73	10.00	58.75	10.00	5.00
4195.16	12.40	7.30	7.71	13.16	57.02	13.37	4.06
1846.54	12.82	5.06	2.53	7.96	36.09	2.96	1.18
44.02	103.35	69.26	32.85	60.47	63.30	22.14	29.20
2692.24	15.39	19.90	5.80	16.67	84.61	23.33	5.00
3738.33	22.46	10.53	8.92	39.33	167.33	16.00	4.67
4774.83	13.04	13.77	8.22	10.00	46.25	7.50	2.50
3735.13	16.96	14.73	7.65	22.00	99.40	15.61	4.06
1041.30	4.91	4.76	1.63	15.38	61.88	7.92	1.36
27.88	28.91	32.31	21.38	69.89	62.26	50.76	33.47

Table VIIIc

Tissue-to-Liver (%)						
Lung	Skin	Muscle	Heart	Kidney	Pancreas	Brain
0.42	0.55	0.22	0.34	1.50	0.25	0.17
NC	NC	NC	NC	NC	NC	NC
0.07	0.27	0.15	0.28	0.76	0.33	0.08
0.25	0.41	0.18	0.31	1.13	0.29	0.12
0.23	0.28	0.11	0.18	0.75	0.17	0.08
92.12	67.26	61.23	58.54	66.34	59.17	69.11
0.31	NC	0.25	0.31	0.86	0.62	0.12
NC	NC	NC	0.37	1.53	0.26	0.07
0.07	0.11	0.16	0.24	1.39	0.24	0.12
0.19	0.11	0.20	0.31	1.26	0.37	0.10
0.16	0.06	0.13	0.07	0.35	0.22	0.03
84.80	57.74	61.78	21.69	27.98	58.33	24.59
0.57	0.74	0.22	0.62	3.14	0.87	0.19
NC	NC	NC	1.05	4.48	0.43	0.12
0.27	0.29	0.17	0.21	0.97	0.16	0.05
0.42	0.51	0.19	0.63	2.86	0.48	0.12
0.29	0.37	0.11	0.42	1.77	0.36	0.07
67.69	72.51	58.81	67.22	61.85	74.00	55.19

Example 4: A Repeat Dose Oral Tablet Administration or Intravenous Infusion

Determination of the pharmacokinetic profile and elimination of Pentamidine (Pentamidine isethionate), following repeat dose administration twice daily for 3 days by oral tablet or once daily (2-hour peripheral intravenous (IV) infusion) for 2 days by infusion to male Beagle dogs. The tissue elimination period studied in this example spans over 56 days. The tablet formulation tested in this example does not contain any absorption or permeation enhancer.

EXPERIMENTAL DESIGN

PENTAMIDINE was administered twice daily by oral tablet administration for 3 days or once daily by 2-hour peripheral intravenous infusion for 2 days, as shown below:

Treatment Group	Dose Route	Compound	Dose Level (mg/kg/day)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Infusion Rate (mL/kg/hr)	Number of Males
1.PENTAMIDINE-Formulation 1	IV ^a	Pentamidine	4	1	4	2	9 ^d
2.PENTAMIDINE-Formulation 2	Oral Tablet ^b	Negative control	N/A	N/A	N/A	N/A	2 ^e
3.PENTAMIDINE-Formulation 3	Oral Tablet ^b	PO Pentamidine	30 ^g	N/A	N/A	N/A	6 ^f

^a 2-hour peripheral intravenous infusion once daily for 2 days.

^b Twice daily (12 hours apart) oral tablet for 3 days.

^d Necropsy/last sample collection from 3 animals at each of the following time-points relative to Day 1: 14, 28 and 56 days post-dose.

^e Necropsy/last sample collection from one animal at each of the following time-points relative to Day 1: 4 and 56 days post-dose.

^f Necropsy/last sample collection from 3 animals at each of the following time-points relative to Day 1: 4 and 56 days post-dose.

^g All doses reported in PENTAMIDINE salt form equivalent (1.74 mg salt for 1 mg freebase). Tablets will be administered at a fixed dose (1x210 mg/animal/occasion, based on target average weight of animals of 7 kg, 30 mg/kg/occasion).

N/A: not applicable

Results

Biodistribution of PENTAMIDINE in organs following PO administration using an instant-release tablet favored the liver, most likely by a first-pass extraction. By normalizing the liver concentrations, one can determine the key organ exposures endured from a PO administration versus an IV administration. As seen below and in figure 9, significantly

lower exposure to the pancreas and kidney, known target organs of pentamidine IV, is observed.

Using the IV administration results of examples 3 and 4, it was possible to confirm the long liver elimination half-life in higher rank animals such as the dog. The elimination half-life from the liver is calculated to be approximately of 28 days, making possible the accumulation and maintenance of therapeutically active concentrations in the liver with minimal systemic exposure.

Example 5: GalN/LPS Fulminant Liver Injury: An Anti-inflammatory, Anti-TNF α , Hepatoprotection model.

To evaluate the anti-inflammatory, anti-TNF α and hepato-protective properties of PENTAMIDINE, a Galactosamine/ Liposaccharide (GalN/LPS) fulminant liver injury model was used. In order to test the biological properties of PENTAMIDINE in a fulminant injury model such as the GalN/LPS, mice and therefore intraperitoneal administration (IP) knowing this would provide best drug exposure to the liver, and mimic as best possible the exposure that a dog or a man would have following oral administration.

Three independent experiments on mice untreated or pretreated with vehicle, compound PENTAMIDINE (isethionate salt) at doses of 25 and 40 mg/kg. PENTAMIDINE is dosed IP 30-minutes prior to the co-treatment with subtoxic doses of hepatotoxin GalN and immune stimulant LPS. Mice were sacrificed at 6.5 hours after GalN/LPS for analysis. The analysis of alanine transaminase (ALT) serum levels and histological observations allow the evaluation of the hepato-protective, anti-TNF- α , anti-inflammatory and/or anti-fibrotic activity.

Results

Serum ALT levels obtained following PENTAMIDINE IP administration of 25 mg/kg or 40 mg/kg are reduced 93% and 97% respectively relative to the treatment with the PBS

vehicle. The results clearly indicate that PENTAMIDINE blocks liver injury (Results: PBS 5,632 +/- 1,223; PENTAMIDINE 25 mg/kg = 402 +/- 105; PENTAMIDINE 40 mg/kg = 177 +/- 44) (see Figure 10-12).

Example 6: Pentamidine Salts in GalN/LPS Fulminant Liver Injury Model: An Anti-inflammatory, Anti-TNF α , Hepatoprotection model.

To evaluate the anti-inflammatory, anti-TNF α and hepatoprotective properties of different pentamidine salt such as pentamidine bis-HCl (penta-HCL) and pentamidine tosylate (penta-Tos), a Galactosamine/ Liposaccharide (GalN/LPS) fulminant liver injury model was used.

Pentamidine HCl and Tosylate were dosed IP 30-minutes prior to the co-treatment with subtoxic doses of hepatotoxin GalN and immune stimulant LPS. Mice were sacrificed at 6.5 hours after GalN/LPS for analysis. The analysis of alanine transaminase (ALT) serum levels and histological observations allow the evaluation of the hepato-protective, anti-TNF- α , anti-inflammatory and/or anti-fibrotic activity.

Results

Results indicate that pentamidine salts also possess the intrinsic activity (see figure 13).

Example 7: Oral bioavailability of pentamidine salts

In this experiment, HCl, isethionate and tosylate salts of pentamidine were administered orally to mice and their PK and tissue accumulations were compared.

PK parameters for pentamidine salt following oral administration of 200 mg/kg in mice.

	Isethionate salt	HCl salt	Tosylate salt
Cmax (uM)	3.06	5.24	0.43
Tmax (hr)	3.33	2.67	6.67

AUCINF (uM*hr)	23.0	158.1	17.8
Cl/F (L/kg/hr)	15.3	5.1	26.4
Vz/F (L/kg)	994	352	1464
t1/2 (hr)	44.5	90.8	54.9
MRT (hr)	52.8	136.0	86.0
F*	3.0	20.7	2.3

* Based on the iv AUC at 5 mg/kg of 19.08

Biodistribution of Pentamidine Salts in mice, 24 hours after PO administration of 200 mg/kg.

		Tissue Concentration (uM)			
	Salt	Kidney	Liver	Lung	Pancreas
Penta-Ise	Diisethionate	5.0	5.6	2.7	1.3
Penta-HCL	HCl	156.3	224.6	43.4	16.6
Penta-Tos	Tosylate	1.9	4.1	0.5	0.5

Different salts of pentamidine have different bioavailability, which may be affected by formulation and/or their intrinsic solubility. (see figure 14).

Example 8: Diabetic and High-Fat Diet Model.

In this experiment, NASH-like pathology is induced in C57BL/6 mice by a single subcutaneous injection of 200 ug streptozotocin 2 days after birth and feeding with high fat diet (HFD, 57 kcal% fat, cat#: HFD32, CLEA Japan, Japan) after 4 weeks of age (STAM mice). Vehicle or pentamidine treatment was administered three times a week from 6 to 9 weeks of age. Vehicle (0.9% NaCl) and PENTAMIDINE were administered by intraperitoneal route in a volume of 10 mL/kg.

Group	No. mice	Mice	Test article	Dose	Volume (mL/kg)	Regimens	Sacrifice (wks)
1	6	STAM	Vehicle	-	10	IP, three times a week, 6wks -9wks	9
2	6	STAM	PENTAMIDINE	5 mg/kg	10	IP, three times a week, 6wks -9wks	9
3	6	STAM	PENTAMIDINE	25 mg/kg	10	IP, three times a week, 6wks -9wks	9

Results:

Results obtained from this experiment indicate a trend for benefice of using PENTAMIDINE in the treatment of NASH. Liver weights of the high dose group was close to normal mice control values (Figure 15) while fibrosis formation showed a trend for reduction in the PENTAMIDINE high dose group (Figure 16).

Example 9: Aromatic Diamidines in GalN/LPS Fulminant Liver Injury Survival Study

In this experiment, the 8-hour survival rate for C57BL/6 mice is evaluated when treated or not with two different aromatic diamidines (pentamidine isethionate(C5) and decamidine isethionate (C10)). Both compounds were administered intraperitoneally at doses close to their MTD 30 minutes prior a GalN/LPS-induced liver injury. Results indicate that aromatic diamidines have potential in hepatoprotection.

	8-hour survival
PBS	0%
PBS/GalN/LPS	0%
PENTAMIDINE (40 mpk)	83%
Decamidine (10 mpk)	100%

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CLAIMS:

1. A method for the targeted treatment of one or more liver conditions comprising the step of orally administering a therapeutically effective amount of at least one diamidine analogue or a pharmaceutically acceptable salt thereof to a human patient in need thereof; wherein the liver condition is liver cancer, liver metastasis, high cholesterol, alcoholic liver disease, cirrhosis, cysts, fatty liver disease (NAFLD), fibrosis, jaundice, primary sclerosing cholangitis (PSC), hemochromatosis, primary biliary cirrhosis or Alpha-1 Antitrypsin Deficiency.
2. The method of claim 1, wherein the levels of the diamidine are increased in the liver relative to the levels in other tissues or organs.
3. The method of claim 1 or 2, wherein the diamidine is pentamidine.
4. The method of claim 1 or 2, wherein the diamidine is decamidine.
5. The method of claim 1 or 2, wherein the diamidine is pentamidine bis(2-hydroxyethanesulfonate), pentamidine isethionate, pentamidine HCl or pentamidine tosylate.
6. The method of claim 1 or 2, wherein the diamidine is pentamidine isethionate.
7. The method of any one of claims 1 to 6 wherein the diamidine is administered at least once daily at a dose of about 1 mg to about 3000 mg.
8. The method of any one of claims 1 to 6 wherein the diamidine is administered at least once daily at a dose of about 1 mg to about 2000 mg.

9. The method of any one of claims 1 to 6 wherein the diamidine is administered at least once daily at a dose of about 50 mg to about 700 mg.
10. The method of any one of claims 1 to 9, wherein the diamidine is administered for more than 15 consecutive days.
11. The method of any one of claims 1 to 9, wherein the diamidine is administered for more than 60 consecutive days.
12. The method of any one of claims 1 to 9, wherein the diamidine is administered for more than 120 consecutive days.
13. The method of any one of claims 1 to 9, wherein the diamidine is administered for more than 365 consecutive days.
14. The method of any one of claims 1 to 13, wherein the diamidine is orally administered as a solid dosage form.
15. The method of any one of claims 1 to 13, wherein diamidine is orally administered as an instant release solid dosage form.
16. The method of any one of claims 1 to 15, wherein the treatment results in a reduced toxicity relative to an equivalent diamidine dose administered parentally.
17. The method of any one of claims 1 to 15, wherein the treatment results in a reduced kidney, heart or pancreas toxicity relative to an equivalent diamidine dose administered parentally.
18. The method of claim 16 or 17, wherein the equivalent diamidine dose is administered intravenously.

19. The method of any one of claims 1 to 18, wherein the liver cancer is intra-hepatic bile duct cancer or hepatocarcinoma.
20. The method of any one of claims 1 to 18, wherein the hepatic metastases are liver dominant cancer metastasis or liver limited cancer metastasis.
21. The method of any one of claims 1 to 18, wherein the hepatic metastases are liver dominant colorectal cancer metastasis.
22. The method of any one of claims 1 to 21, wherein the diamidine is administered in combination with standard chemotherapy.
23. The method of any one of claims 1 to 21, wherein the diamidine is administered in combination with cisplatin, oxaliplatin, mitomycin C, melphalan, carmustine, adriamycin, paclitaxel, docetaxel, 5-fluorouracil, bevacizumab, cetuximab, capecitabine, folinic acid (also known as leucovorin), ionizing irradiation, bleomycin, carboplatin, irinotecan, or gemcitabine.
24. The method of any one of claims 1 to 21, wherein further agent is carboplatin or gemcitabine.
25. The method of any one of claims 1 to 18, wherein the liver condition is alcoholic liver disease.
26. The method of any one of claims 1 to 18, wherein the liver condition is acute alcoholic hepatitis).
27. The method of any one of claims 1 to 18, wherein the liver condition is primary biliary cirrhosis.

28. The method of any one of claims 1 to 18, wherein the liver condition is fatty liver disease (NAFLD)
29. The method of any one of claims 1 to 18, wherein the liver condition is Alpha-1 Antitrypsin Deficiency.
30. The method of any one of claims 1 to 18, wherein the liver condition is primary sclerosing cholangitis (PSC).
31. The method of any one of claims 1 to 18 and 25 to 30, wherein the diamidine is administered in combination with a further agent indicated for the treatment of the one or more liver condition.
32. Oral use of a therapeutically effective amount of at least one diamidine analogue or a pharmaceutically acceptable salt thereof for the targeted treatment of one or more liver conditions in a human patient in need thereof; wherein the liver condition is, liver cancer, liver metastasis, high cholesterol, alcoholic liver disease, cirrhosis, cysts, fatty liver disease (NAFLD), fibrosis, jaundice, primary sclerosing cholangitis (PSC), hemochromatosis, primary biliary cirrhosis or Alpha-1 Antitrypsin Deficiency.
33. The use of claim 32, wherein the levels of the diamidine are increased in the liver relative to the levels in other tissues or organs.
34. The use of claim 32 or 33, wherein the diamidine is pentamidine.
35. The use of claim 32 or 33, wherein the diamidine is decamidine.

36. The use of claim 32 or 33, wherein the diamidine is pentamidine bis(2-hydroxyethanesulfonate), pentamidine isethionate, pentamidine HCl or pentamidine tosylate.
37. The use of claim 32 or 33, wherein the diamidine is pentamidine isethionate.
38. The use of any one of claims 32 to 37 wherein the diamidine is used at least once daily at a dose of about 1 mg to about 3000 mg.
39. The use of any one of claims 32 to 37 wherein the diamidine is used at least once daily at a dose of about 1 mg to about 2000 mg.
40. The use of any one of claims 32 to 37 wherein the diamidine is used at least once daily at a dose of about 50 mg to about 700 mg.
41. The use of any one of claims 32 to 40, wherein the diamidine is used for more than 15 consecutive days.
42. The use of any one of claims 32 to 40, wherein the diamidine is used for more than 60 consecutive days.
43. The use of any one of claims 32 to 40, wherein the diamidine is used for more than 120 consecutive days.
44. The use of any one of claims 32 to 40, wherein the diamidine is used for more than 365 consecutive days.
45. The use of any one of claims 32 to 44, wherein the diamidine is used as a solid dosage form.

46. The use of any one of claims 1 to 13, wherein diamidine is used as an instant release solid dosage form.
47. The use of any one of claims 32 to 46, wherein the treatment results in a reduced toxicity relative to an equivalent diamidine dose administered parentally.
48. The method of any one of claims 32 to 46, wherein the treatment results in a reduced kidney, heart or pancreas toxicity relative to an equivalent diamidine dose administered parentally.
49. The use of claim 47 or 48, wherein the equivalent diamidine dose is administered intravenously.
50. The use of any one of claims 32 to 49, wherein the liver cancer is intra-hepatic bile duct cancer or hepatocarcinoma.
51. The use of any one of claims 32 to 49, wherein the hepatic metastases are liver dominant cancer metastasis or liver limited cancer metastasis.
52. The use of any one of claims 32 to 49, wherein the hepatic metastases are liver dominant colorectal cancer metastasis.
53. The use of any one of claims 32 to 52, wherein the diamidine is used in combination with standard chemotherapy.
54. The use of any one of claims 32 to 52 wherein the diamidine is used in combination with cisplatin, oxaliplatin, mitomycin C, melphalan, carmustine, adriamycin, paclitaxel, docetaxel, 5-fluorouracil, bevacizumab, cetuximab, capecitabine, folinic acid (also known as leucovorin), ionizing irradiation, bleomycin, carboplatin, irinotecan, or gemcitabine

55. The use of any one of claims 32 to 52, wherein further agent is carboplatin or gemcitabine.
56. The use of any one of claims 32 to 49, wherein the liver condition is alcoholic liver disease.
57. The use of any one of claims 32 to 49, wherein the liver condition is acute alcoholic hepatitis.
58. The use of any one of claims 32 to 49, wherein the liver condition is primary biliary cirrhosis.
59. The use of any one of claims 32 to 49, wherein the liver condition is fatty liver disease (NAFLD)
60. The use of any one of claims 32 to 49, wherein the liver condition is Alpha-1 Antitrypsin Deficiency.
61. The use of any one of claims 32 to 49, wherein the liver condition is primary sclerosing cholangitis (PSC).
62. The use of any one of claims 32 to 49 and 56 to 61 wherein the diamidine is used in combination with a further agent indicated for the treatment of the one or more liver condition.

Figure 1

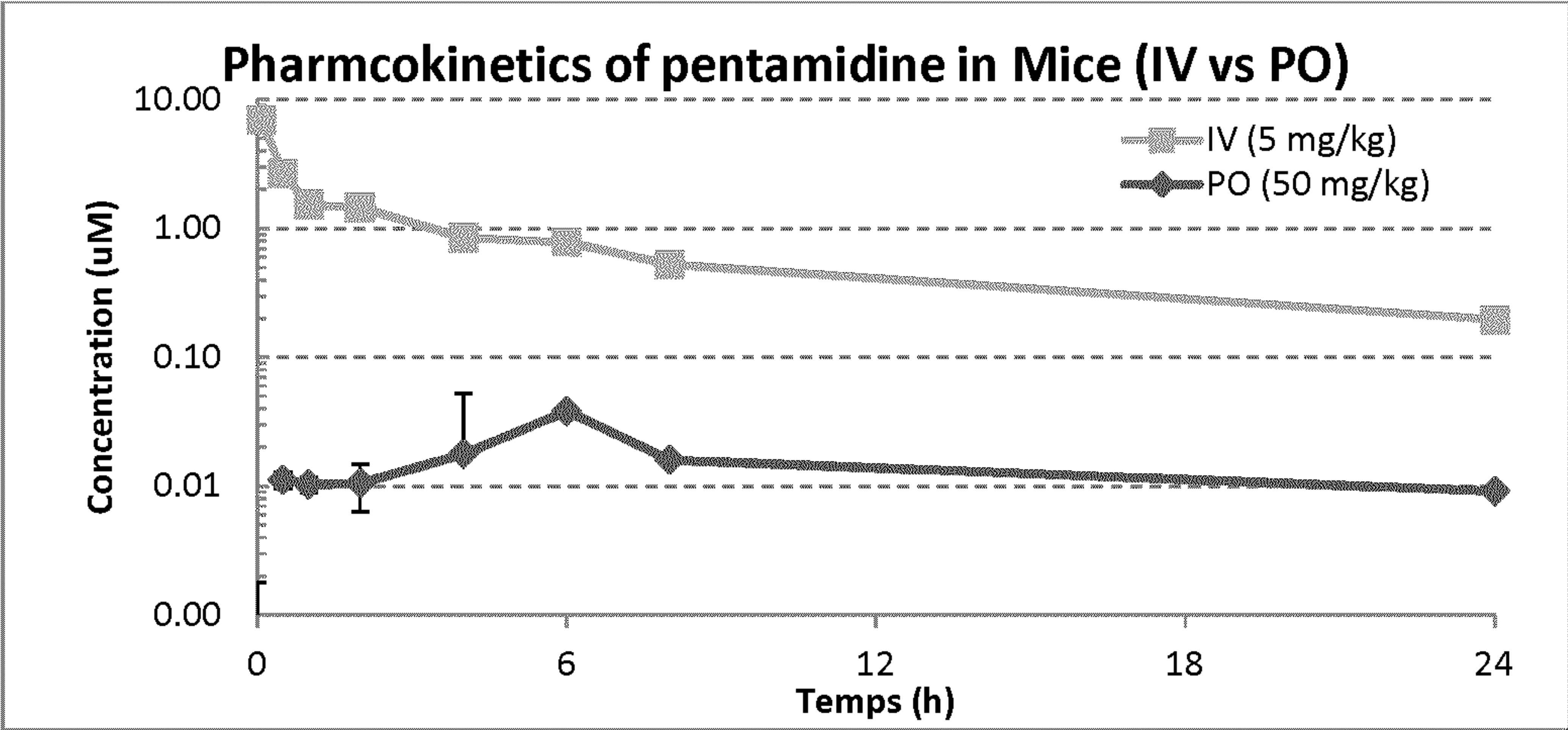
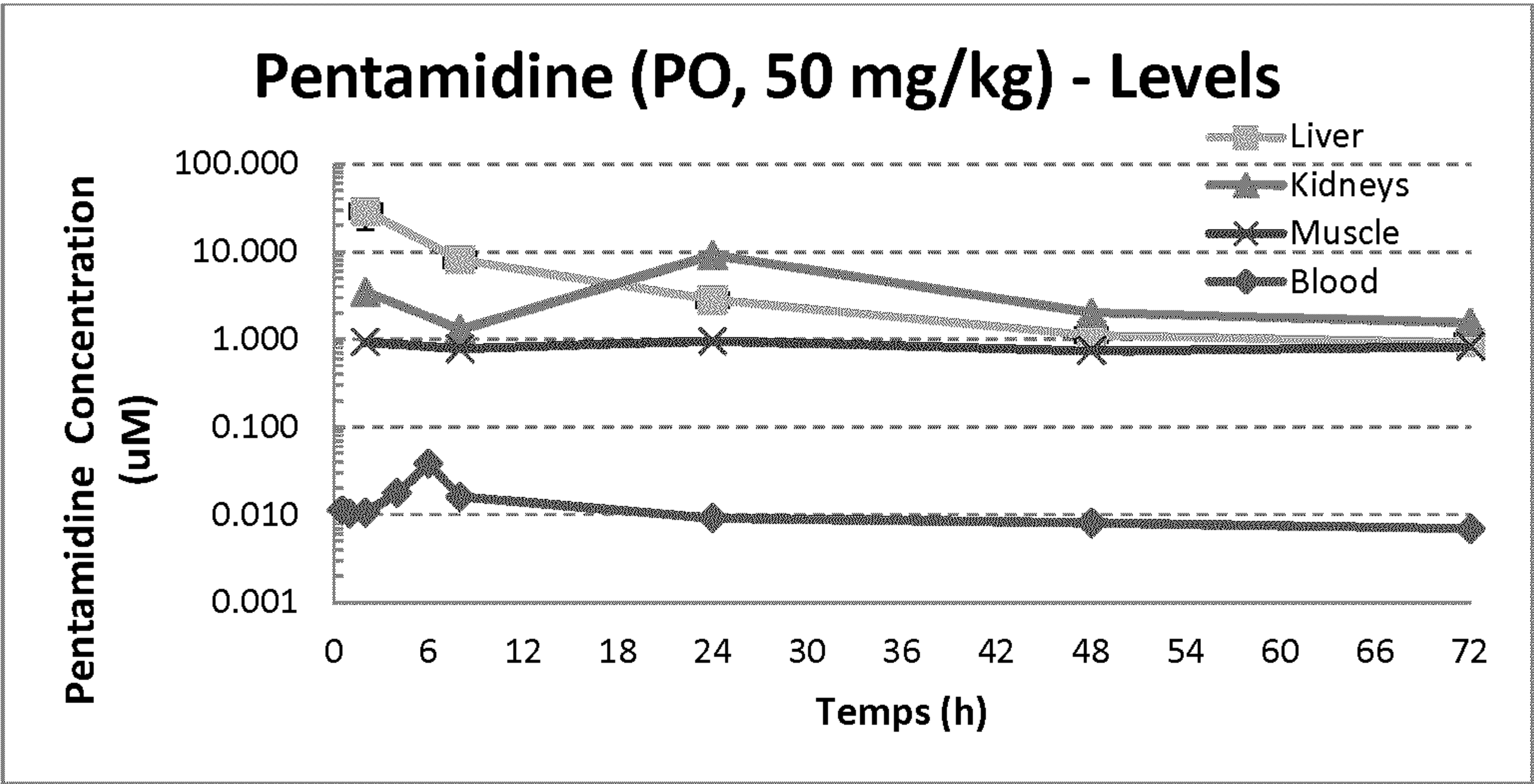
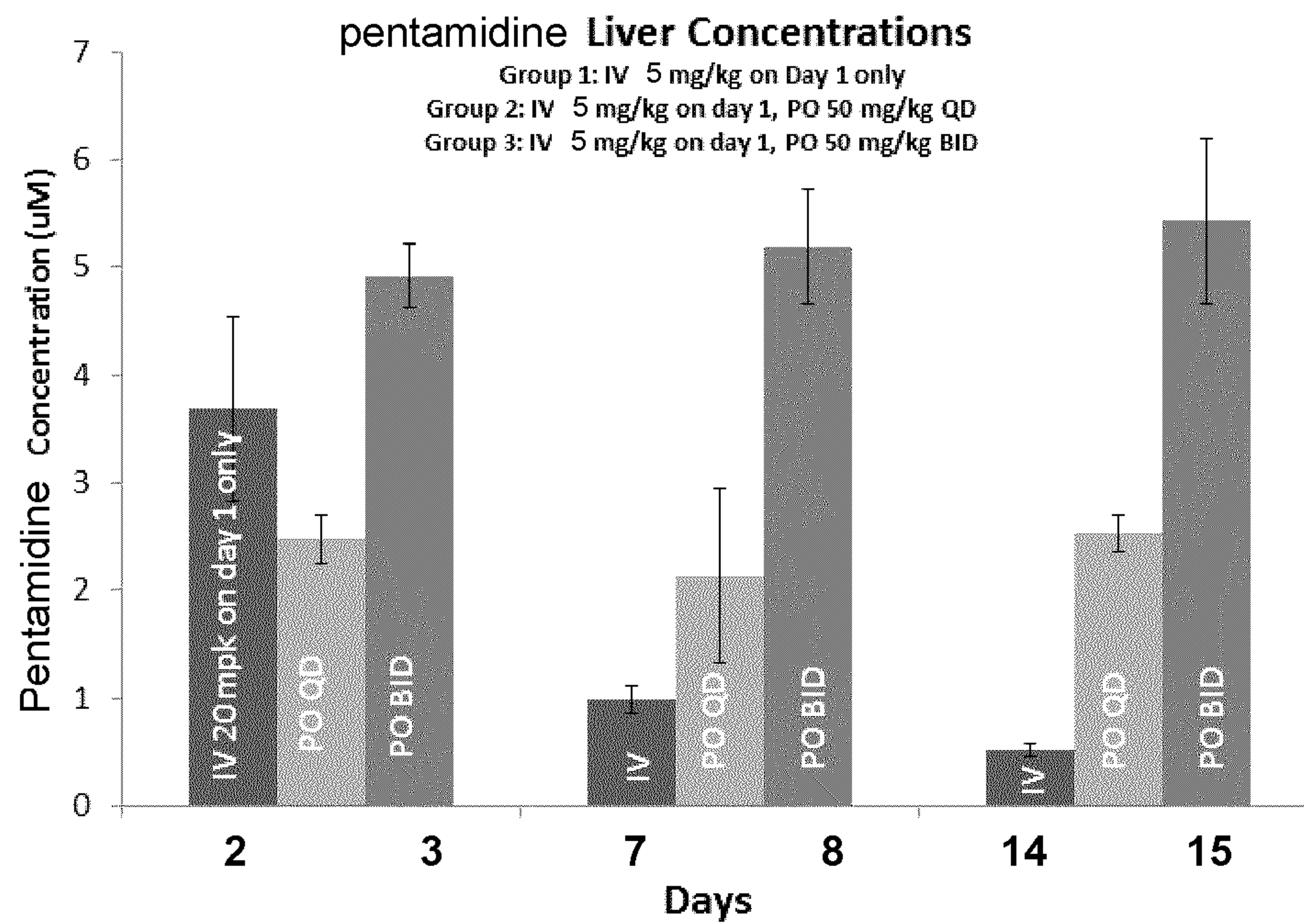
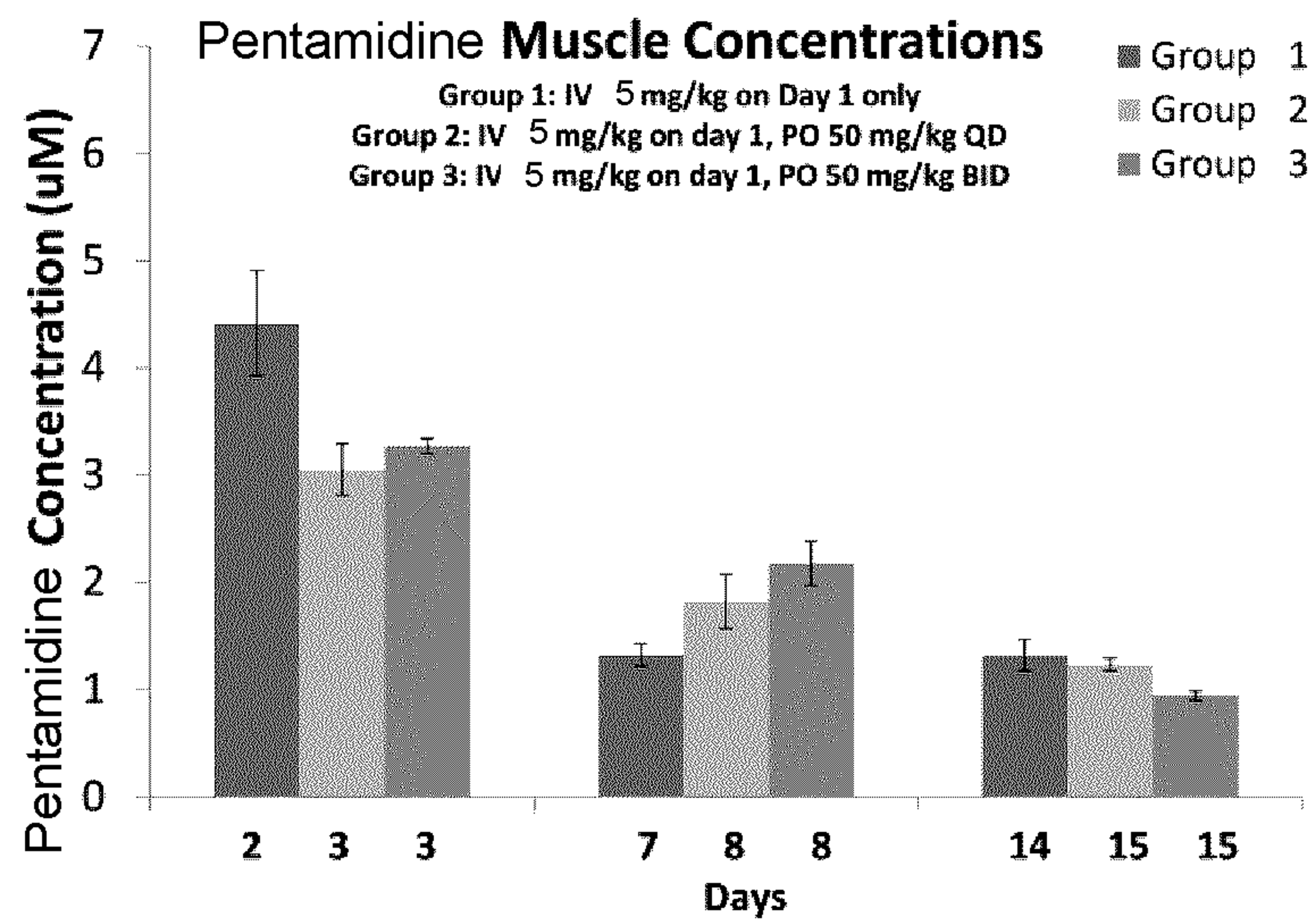


Figure 2



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Figure 3Figure 4

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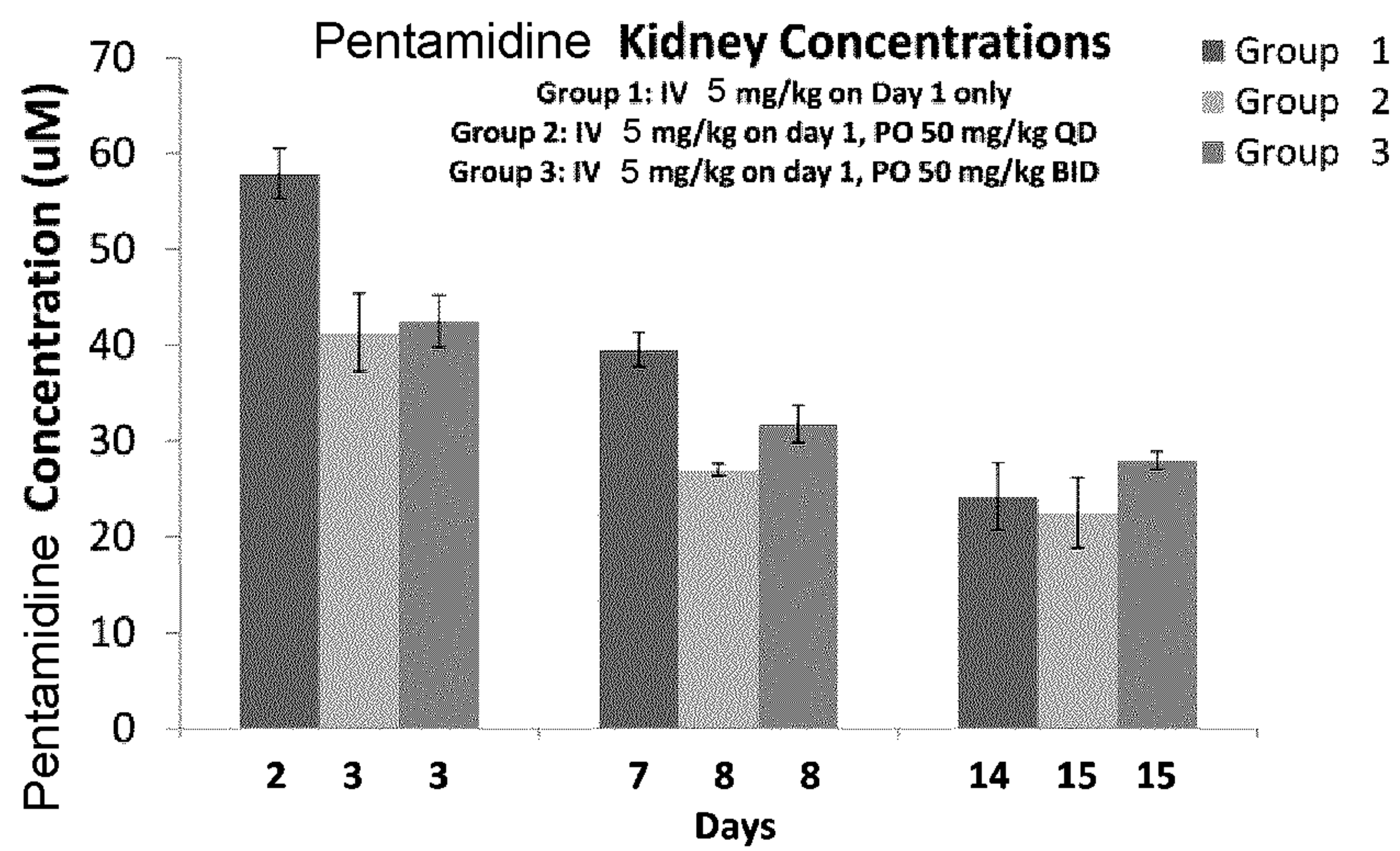
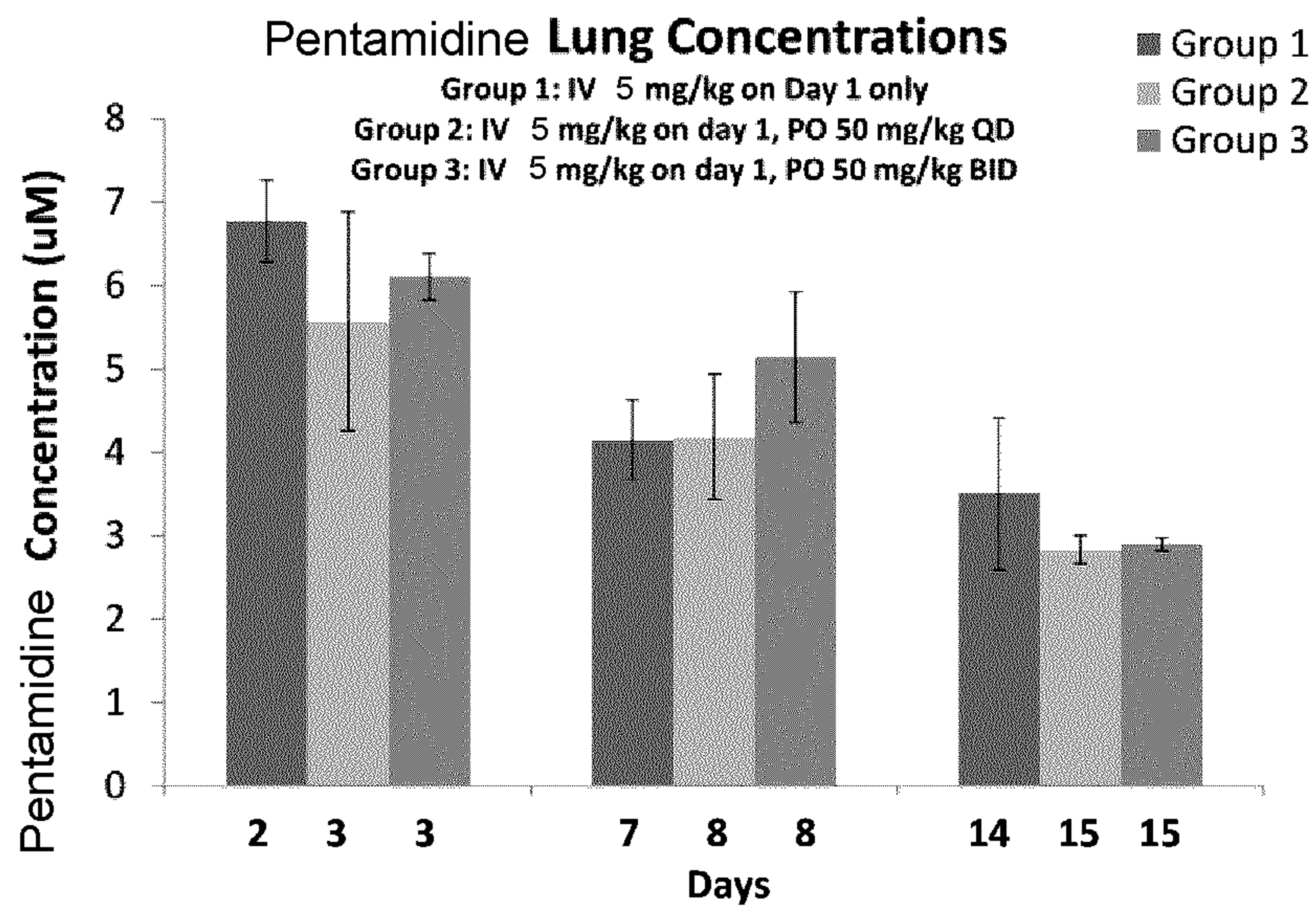
Figure 5Figure 6

Figure 7

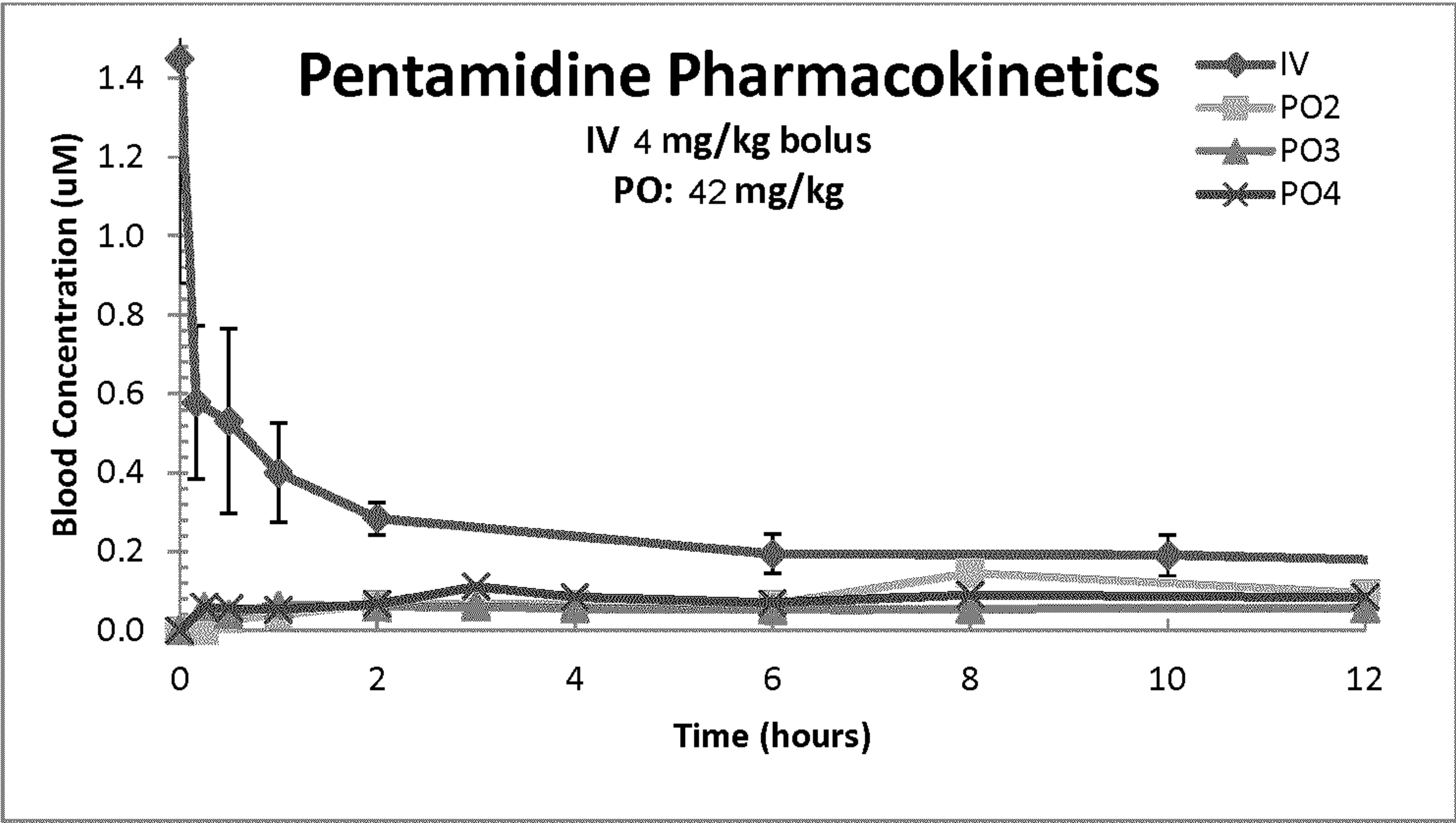
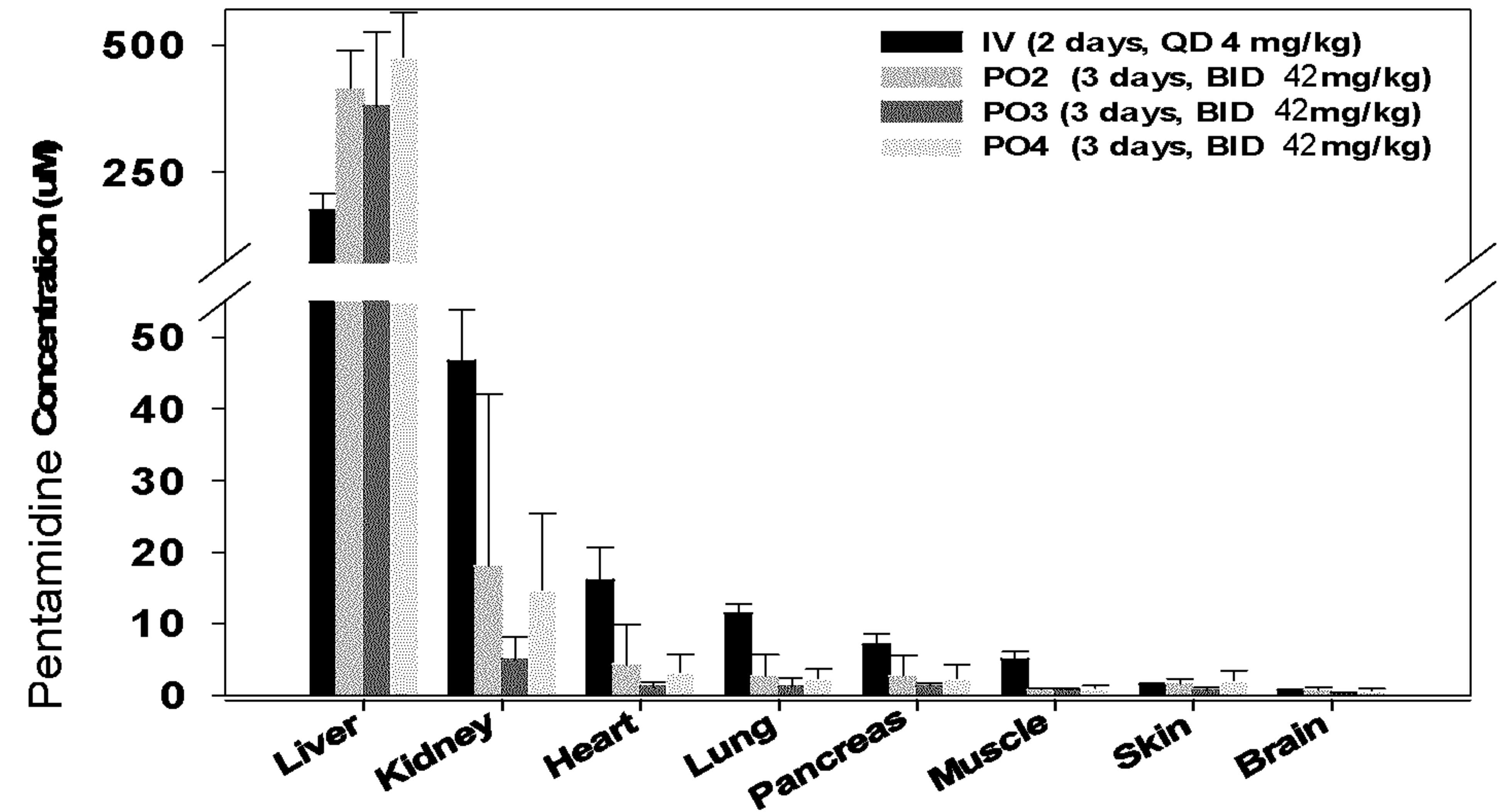


Figure 8



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Figure 9

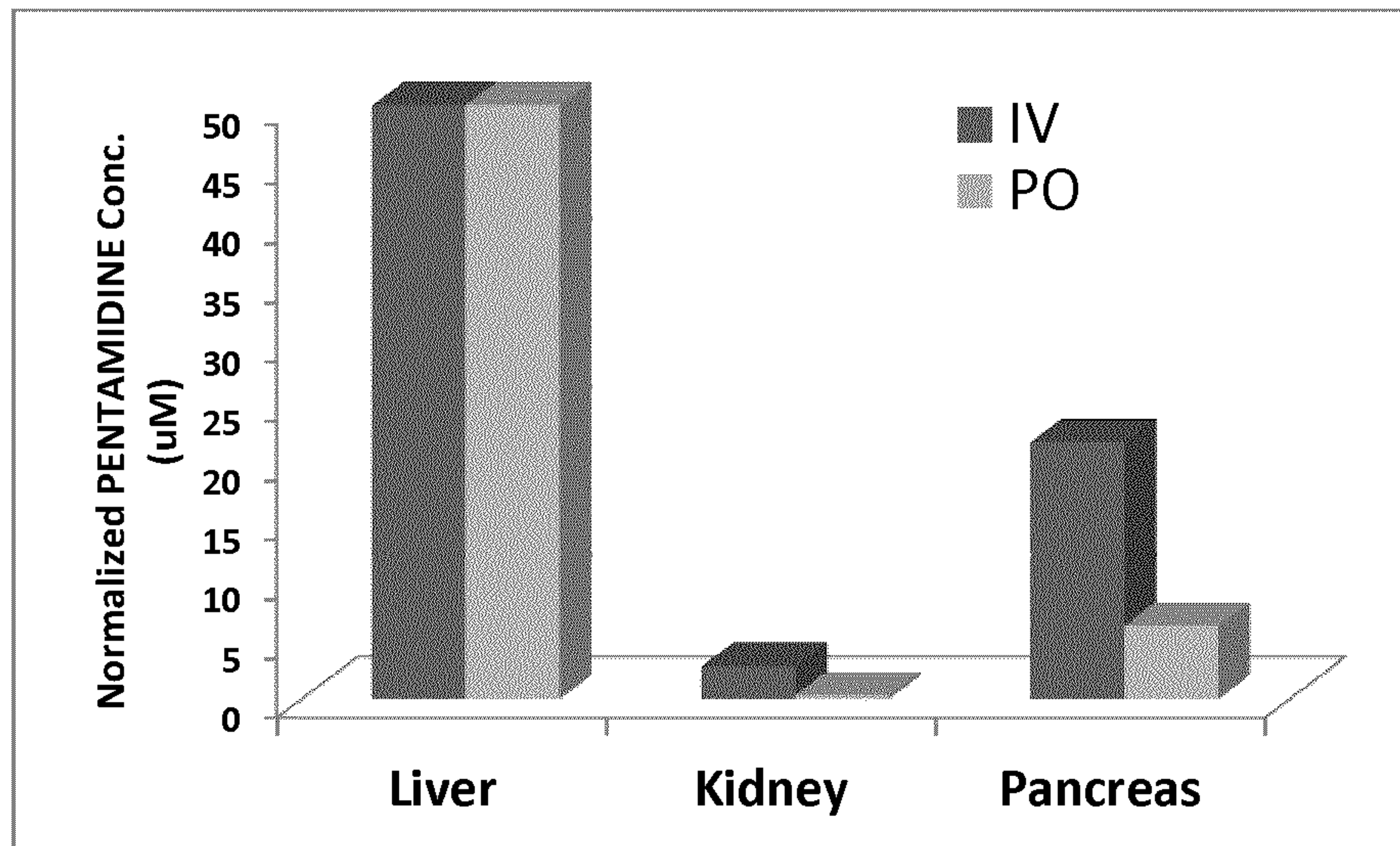
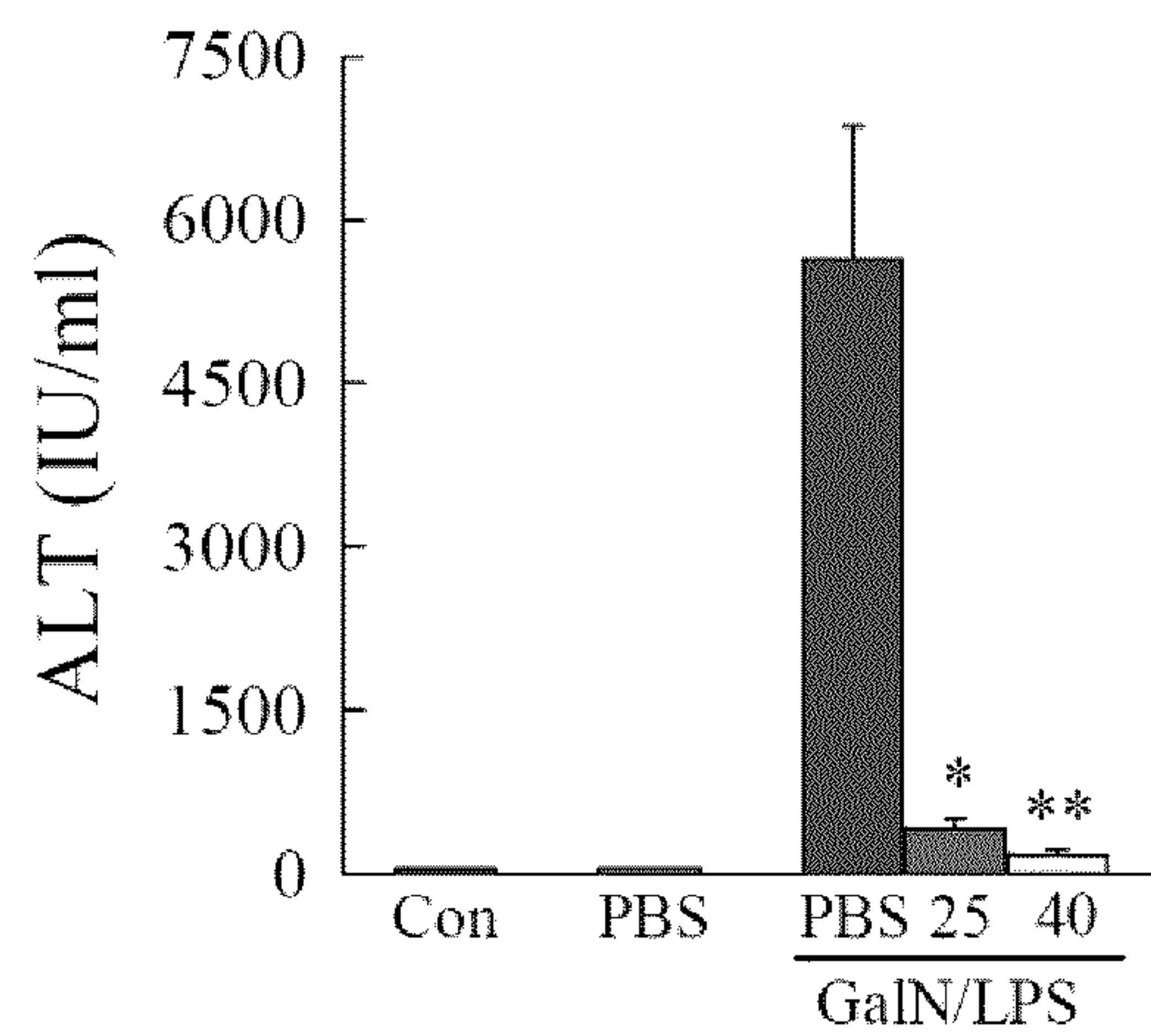


Figure 10



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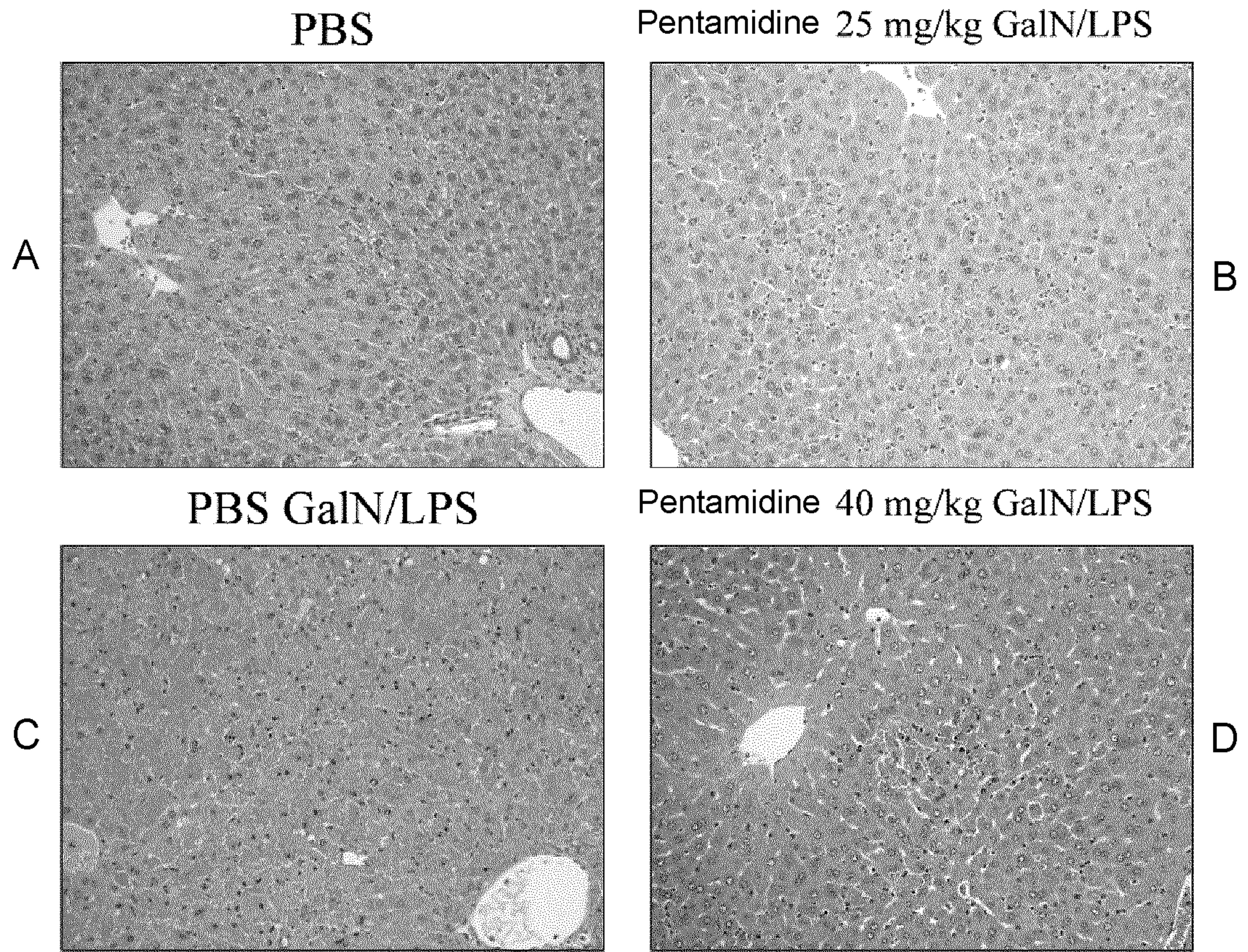
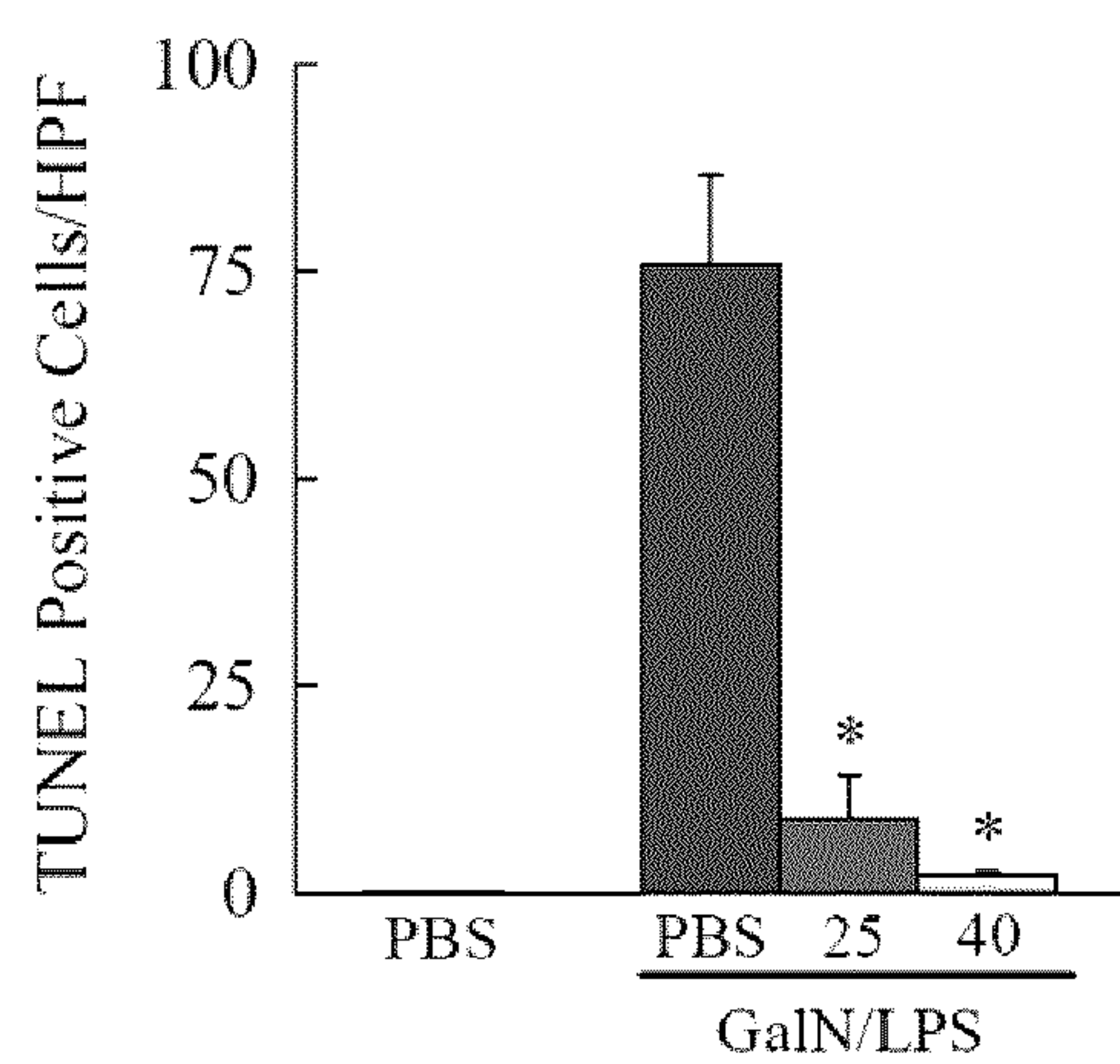
Figure 11Figure 12

Figure 13

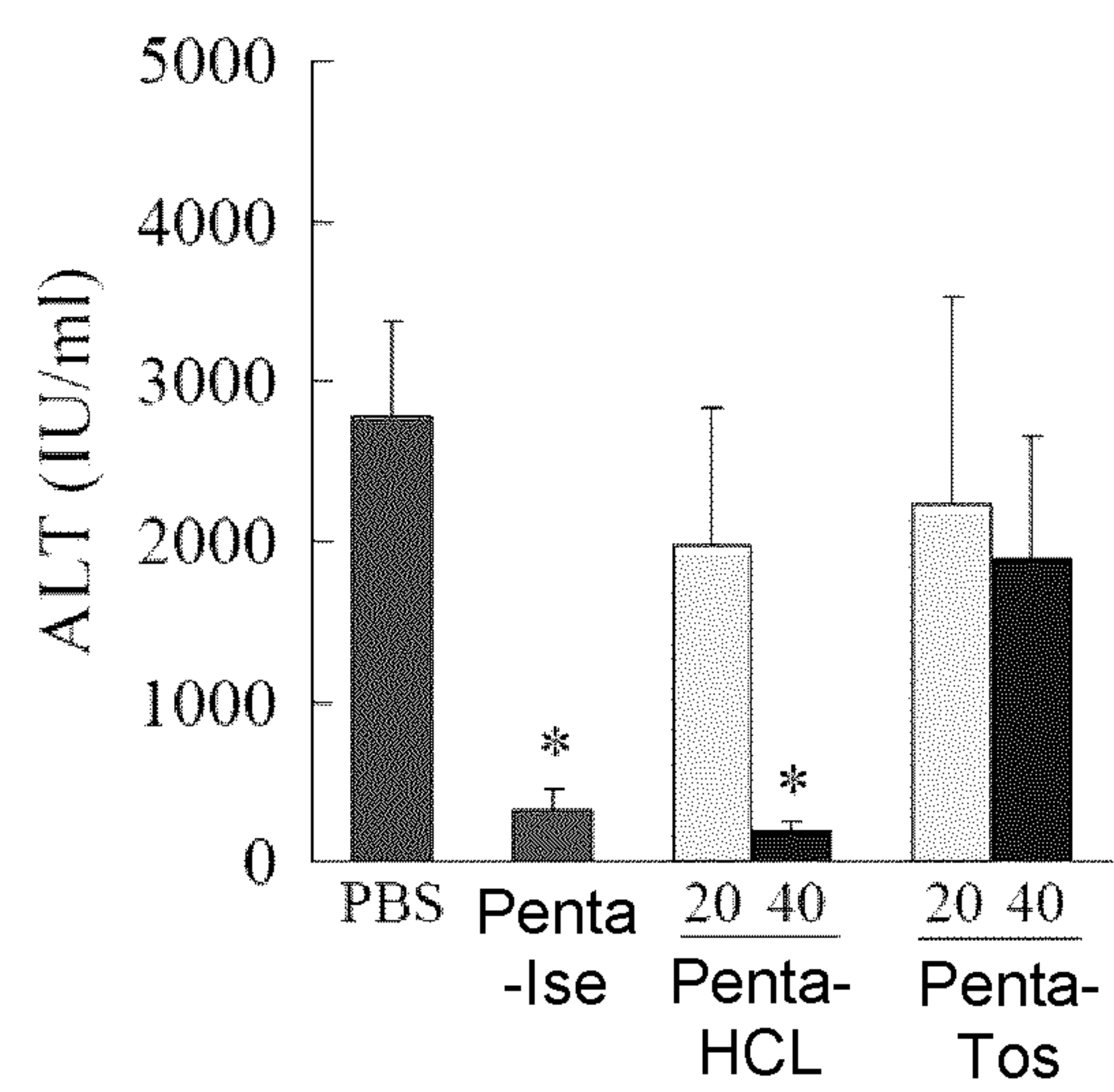


Figure 14

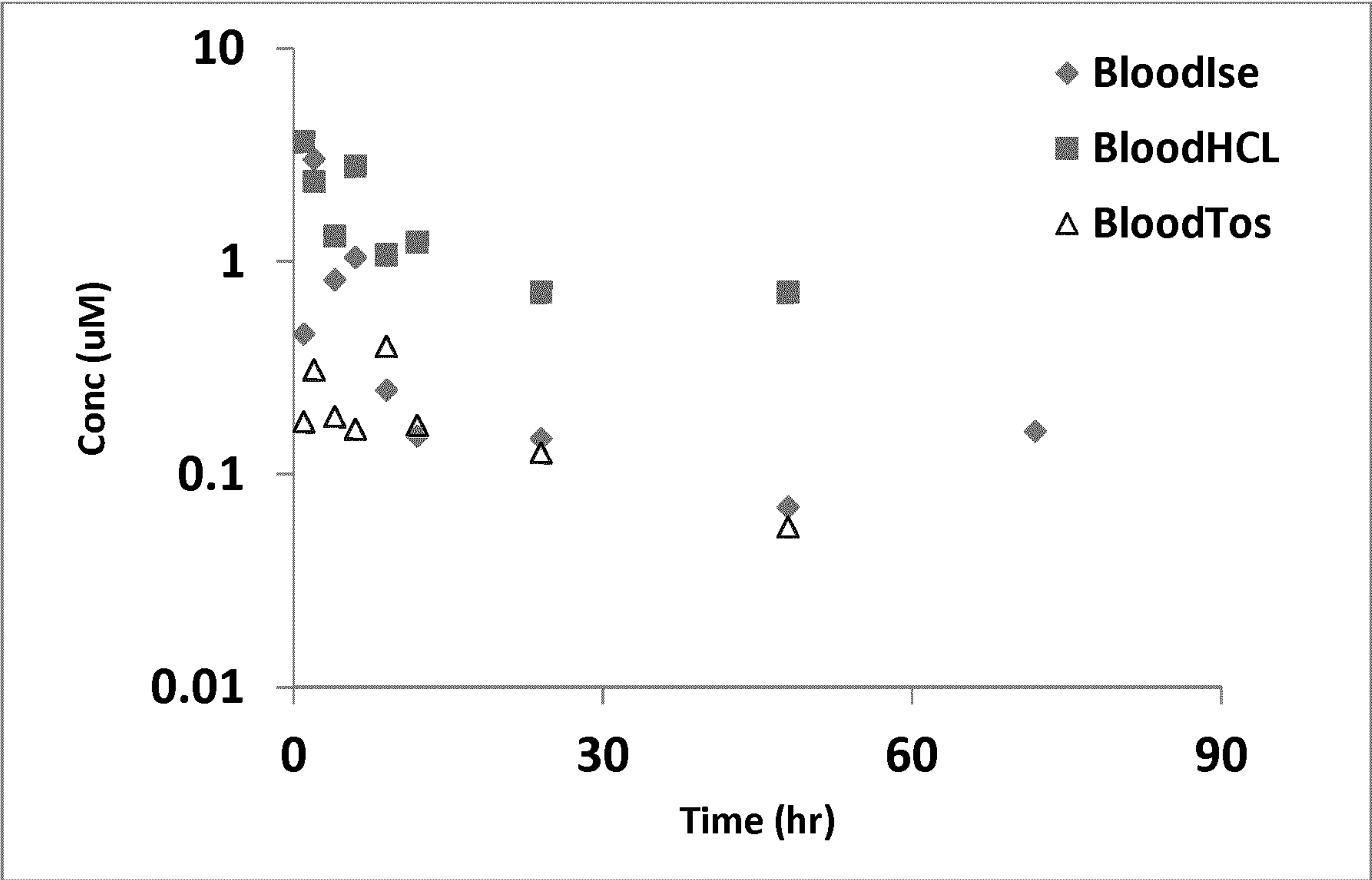


Figure 15

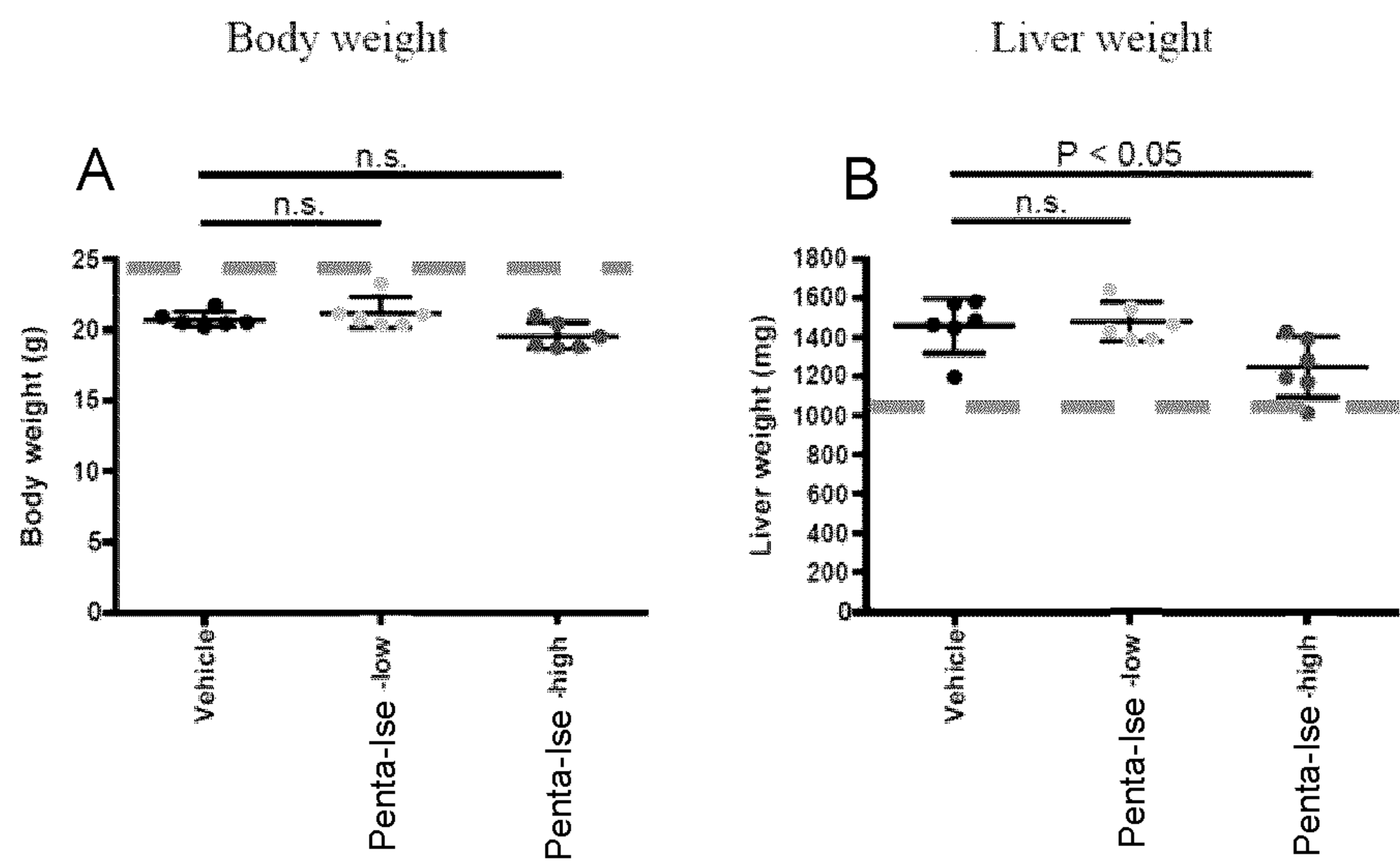


Figure 16

