Compositions Useful for Reducing Nephrotoxicity and Methods of Use Thereof

Background of the Invention

Numerous drugs and other substances are known to be nephrotoxic and can cause renal failure through a variety of mechanisms including direct toxicity to the renal tubules, allergic interstitial nephritis, and crystallization of the drug within the renal tubules, which can lead to acute oliguric renal failure. Nephrotoxic drugs include anticancer agents such as cisplatin, methotrexate, and doxyrubicin, non-steroidal antiinflammatories (NSAIDS), such as COX-2 inhibitors, antibiotics (e.g., aminoglycosides, amphotericin) antivirals (e.g., acyclovir, indinivir), acetylcholinesterase inhibitors, angiotensin II receptor blockers (ARBs), lithium and radiographic contrast media.

A need exists to reduce renal damage caused by nephrotoxic drugs.

Summary of the Invention

In one aspect, there is provided a pharmaceutical composition comprising a substituted cyclodextrin, a nephrotoxic anticancer drug and a pharmaceutically acceptable carrier, said cyclodextrin being present in an amount effective for substantially inhibiting the nephrotoxic effect of said drug, and wherein the molar ratio of drug: cyclodextrin is greater than 1:1.

In another aspect, there is provided the use of a pharmaceutically active anticancer compound that has a nephrotoxic effect, a substituted cyclodextrin and a pharmaceutically acceptable carrier in the manufacture of a medicament for reducing the nephrotoxic effect, a substituted cyclodextrin and a pharmaceutically acceptable carrier in the manufacture of a medicament for reducing the nephrotoxic effect of said pharmaceutically anticancer active compound, said cyclodextrin being present in an amount effective for substantially reducing said nephrotoxic effect of said pharmaceutically active compound, and wherein the molar ratio of anticancer drug: cyclodextrin is greater than 1:1.

In yet another aspect, there is provided the use of a substituted cyclodextrin in the manufacture of a first pharmaceutical composition for inhibiting nephrotoxicity associated with administration of a nephrotoxic inducing anticancer drug, wherein said first pharmaceutical composition is to be concurrently administered with a second pharmaceutical composition comprising the nephrotoxic inducing anticancer drug, and wherein the molar ratio of drug: cyclodextrin is greater than 1:1.

In yet another aspect, there is provided the use of a therapeutically effective amount of the composition as defined above in the manufacture of a medicament for treating the symptoms associated with multiple sclerosis in a patient in need thereof.

In yet another aspect, there is provided the use of a therapeutically effective amount of the composition as defined above in the manufacture of a medicament for treating cancer in a host in need thereof.

In yet another aspect, there is provided the use of a therapeutically effective amount of the composition as defined above in the manufacture of a medicament for inhibiting the growth of cancer in a host in need thereof.

In yet another aspect, there is provided the use of a therapeutically effective amount of the composition as defined above in the manufacture of a medicament for treating an autoimmune disorder in a host in need thereof.

Disclosed are compositions and methods to reduce renal damage caused by nephrotoxic drugs. Compositions comprising an anionically substituted oligosaccharide, a nephrotoxic drug and a pharmaceutically acceptable carrier, where the oligosaccharide is present in an amount effective for substantially inhibiting the nephrotoxic effect of the drug are also disclosed.

Also provided are compositions having reduced nephrotoxic effect comprising a pharmaceutically active compound having nephrotoxic effect and a polyanionic oligosaccharide where the oligosaccharide is present in an amount effective to substantially reduce the nephrotoxic effect of the pharmaceutically active compound.

Also disclosed herein are methods of reducing the nephrotoxic effect of a pharmaceutically active compound comprising contacting the compound with a polyanionic oligosaccharide. Additionally, methods are disclosed for inhibiting nephrotoxicity associated with a nephrotoxic drug, the method comprising administering a pharmaceutical composition comprising a cyclic polysaccharide sulfate, the nephrotoxic inducing drug and optionally a pharmaceutically acceptable carrier.

Brief Summary of the Figures

Figure 1 shows solubility study results with methotrexate (MTX) and captisol in aqueous acidic solution.

Figure 2 shows renal pathology scores indicative of total kidney damage in myelin-oligodendrocyte-glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE) mouse model following treatment with MTX and MTX + captisol.

Figure 3 shows clinical scores after treatment with MTX or MTX + captisol in EAE mice.

Figure 4 shows renal pathology scores in kidney sections after single bolus intravenous MTX with or without concurrent captisol at different molar ratios in normal mice.

Figure 5 shows renal pathology scores in the kidney tissue of mice 24 and 48 hours after treatment with MTX with or without concurrent captisol at different molar ratios.

Figure 6 shows the mean pathology scores in a doxorubicin induced nephrotoxic model for each treatment group at the level of superficial renal cortex.

Figure 7 shows the renal pathology scores for individual mice treated with doxorubicin or doxorubicin + captisol at the level of superficial renal cortex.

Figure 8 shows the mean pathology scores in a doxorubicin induced nephrotoxic model for each treatment group at the level of deep renal cortex + outer medulla.

Figure 9 shows the renal pathology scores for individual mice treated with doxorubicin or doxorubicin + captisol at the level of deep renal cortex + outer medulla.

Figure 10 shows the mean scores at the level of the superficial cortex in cisplatin and cisplatin + captisol treated groups.

Figure 11 shows the pathology scores in a cisplatin induced nephrotoxic model of individual mice in each treatment group at the level of the superficial renal cortex.

Figure 12 shows the mean scores at the level of the deep cortex and outer medulla in cisplatin and cisplatin + captisol treated groups.

Figure 13 shows pathology scores in a cisplatin induced nephrotoxic model of individual mice in each treatment group at the level of the deep renal cortex and outer medulla.

Detailed Description of the Invention

The compositions of the present invention typically comprise an anionically substituted oligosaccharide, a nephrotoxic drug and typically a pharmaceutically acceptable carrier or other excipient commonly used in the art. The oligosaccharide is present in an amount effective to substantially inhibit the nephrotoxic effect of the drug. In one embodiment, the oligosaccharides are substituted with polar or charged moieties, such as cationic or anionic substituents. In one example, the anionically substituted oligosaccharide is a polyanionic oligosaccharide comprising a cyclodextrin having one or more anionic substituents selected from the group consisting of sulfonate, sulfate, carboxylate, phosphonate and phosphate. In another embodiment, the oligosaccharide is a cyclic polysaccharide sulfate, preferably an α, β or γ-cyclodextrin sulfate.

The present invention also provides compositions having reduced nephrotoxic effect comprising a pharmaceutically active compound having nephrotoxic inducing effect and a polyanionic oligosaccharide. Nephrotoxic as used herein means toxic or destructive to the kidney, or any of its components.

Substituted Oligosaccharides

Substituted oligosaccharides generally refer to oligosaccharides having at least one substituent per molecule, preferably a charged or polar substituent. The oligosaccharides are preferably saccharides of from about 5 to about 10 sugar units and have molecular weights, when unsubstituted, from about 650 to about 1300. Where the oligosaccharide is anionically substituted, it is generally preferred that the substituents be selected from the group consisting of sulfonate, sulfate, carboxylate, phosphonate and phosphate groups and combinations thereof. The substituents are preferably present in the molecule to an extent of from about 0.5 to about 3 substituents per sugar unit. Especially preferred compositions are those based on oligosaccharides having about 1 sulfonate substituent per sugar unit. Other preferred compositions are based on oligosaccharides having from about 2 to about 3 substituents per sugar unit, wherein the substituents comprise sulfate, sulfonate and/or

Oligosaccharides are chains of several sugar units such as glucose units, connected through glycosidic oxygen atoms. As used herein, the prefix "oligo" indicates an intermediate number of sugar or saccharide units, as compared to a monomeric sugar unit of one, or at most two as in sucrose, and a polysaccharide having twenty or more of sugar units and high molecular weight. While all such oligosaccharides are believed to be operable within the scope of the present invention, the oligosaccharides hereof preferably have about 5 to about 10 saccharide units per molecule. This range corresponds to unsubstituted saccharides having molecular weights ranging from about 650 to about 1300. Oligosaccharides having from about 5 to about 10 saccharide units per molecule are sometimes referred to herein as "simple" or "low molecular weight" oligosaccharides. Oligosaccharides are usually obtained by procedures of degradation of starches or cellulose which result in oligosaccharide fragments in a broad range of sizes.

A somewhat related family of materials are the glycosaminoglycans. They are structures comprising a polysaccharide skeleton, modified by a variety of substituents containing nitrogen, sulfur and oxygen atoms, and comprising various segments such as glucosamines, iduronates, glucuronates and the like. Their structures are variable between different samples of the same name group, such as the chondroitans, dermatans, hyaluronic acid, heparan sulfates, and heparins. Each family is known to be heterogenous, *i.e.*, mixtures of compositions. Their molecular weight ranges generally between 10,000 and 25,000.

Substituted oligosaccharides, and, in particular, simple and low molecular weight oligosaccharides with polar or charged substituents, possess the ability to protect the kidneys from the nephrotoxic effect of certain classes of drugs. Anionically substituted cyclodextrins are preferred, at least in part, because of the relative uniformity and ease of production of such compounds, thought other polar substituents such as OH may be used.

Anionic substituents include, by way of example, those described in U.S. Patent No. 3,426,011. Oligosaccharides may be of the general formula:

$$\text{oligosaccharide-}[(\text{O-R-Y})^{-}\ (\text{Me})^{+}]_n$$

where R is selected from the group consisting of straight chained or branched $C_{1\text{-}10}$ alkyl, alkenyl or alkynyl; $C_{3\text{-}8}$ cycloalkyl and $C_{3\text{-}8}$ aryl, each ring optionally containing 1 or more heteroatoms selected from S, N and O; and each of the

aforementioned groups is optionally substituted with halo (i.e., F, Cl, Br, I) or hydroxyl;

Y is an acid group such as OH, COOH, SO_3, SO_4, PO_3H or PO_4, or a phosphorous, phosphinous, phosphonic, phosphinic, thiophosphonic, thiophosphinic and sulfonic acid;

Me is a pharmaceutically acceptable anion or cation, such as lithium, sodium, potassium, calcium, magnesium, or aluminum, or an organic primary, secondary, or tertiary amine such as methylamine, dimethylamine, trimethylamine, triethylamine, diethylamine, ethylamine, tributlyamine, pyridine, N,N-dimethylaniline, N-methylpiperidine, N-methylmorpholine, dicyclohexylamine, procaine, dibenzylamine, N,N-dibenzylphenethylamine, 1-ephenamine, and N,N'-dibenzylethylenediamine, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like; and

n is the number of substituents per oligosaccharide, each of which is independently selected, i.e., each substituent may be the same or different. "N" will be a whole number greater than 1, the upper limit dependent upon the particular oligosaccharide. In a population of oligosaccharides, it will be understood that n will represent the average number of substituents per molecule.

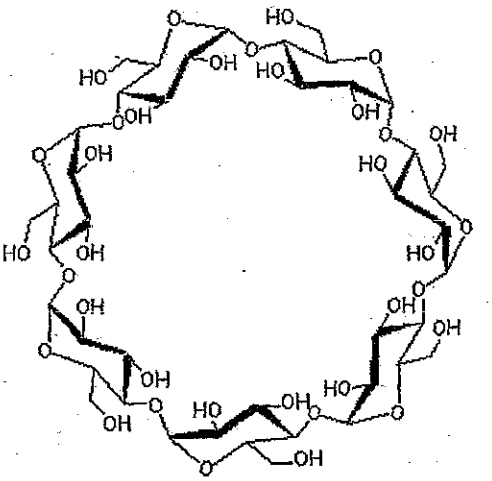
According to one embodiment, R is C_{1-10} alkyl, preferably C_{1-4} alkyl selected from methyl, ethyl, propyl and butyl, each optionally substituted with halo or hydroxyl. Specifically preferred are oligosaccharides where in one or more groups, Y is SO_3. The resulting preferred, polyanionically substituted oligosaccharides have molecular weights of from about 1600 to about 4000.

Cyclodextrins

In a preferred embodiment, the oligosaccharides are cyclic polysaccharides, preferably cyclodextrins, and more preferably derivatized cyclodextrins.

Cyclodextrins (also referred to as "CD" or "CDs") are cyclic oligosaccharides consisting of at least six glucopyranose units. Although CDs with up to twelve glucopyranose units are known, only the first three homologs have been studied extensively, α, β, and γ, having 6, 7 and 8 glucopyranose units, respectively. For example, the β-cyclodextrin molecule is made up of seven α-1,4-linked glucopyranose units which form a cone-shaped molecule having a hydrophilic outer surface and a lipophilic cavity in the center. It is believed that cyclodextrins exist as

conical shaped molecules with the primary hydroxyls situated at the small end of the cone and the secondary hydroxyls situated at the large opening to the cone.



β-Cyclodextrin

Topographically, the CDs may be represented as a torus, the upper rim of which is lined with primary -CH_2OH groups, and the lower rim with secondary hydroxyl groups. Coaxially aligned with the torus is a channel-like cavity of about 5, 6 or 7.5 A.U. diameter for the α, β, and γ -CDs, respectively. These cavities make the cyclodextrins capable of forming inclusion compounds with hydrophobic guest molecules of suitable diameters.

A reasonably large number of CD derivatives have been prepared and described in the literature. In general, these chemically modified CDs are formed by reaction of the primary or secondary hydroxyl groups attached to carbons 2, 3 or 6, without disturbing the α (1 → 4) hemiacetal linkages. A review of such preparations is given in Croft et al., (*Tetrahedron* (1983) 39(9):1417-1474), incorporated herein by reference. Substitution via the hydroxyl groups on the glucopyranose units would include up to 18 for α-CD; 21 for β-CD; and 24 for γ-CD. The cyclodextrins may be selected from dextrins of the formula:

$$\text{cyclodextrin-}[(\text{O-R-Y})^{-}\ (\text{Me})^{+}]_n$$

where R, Y, Me and n are as described above. As will be apparent, n is 1 to 18 for α-CD; 1 to 21 for β-CD; and 1 to 24 for γ-CD.

Preferably, the cyclodextrin will have one or more substituents selected from the group consisting of hydroxyl, sulfonate, sulfate, carboxylate, phosphonate and phosphate. According to one embodiment, R is straight chained or branched C_{1-10} alkyl, preferably C_{1-4} alkyl selected from methyl, ethyl, propyl and butyl, each optionally substituted with halo or hydroxyl. Specifically preferred are oligosaccharides where in one or more groups, Y is SO_3.

Preferred CDs are sulfate or sulfonate derivatives of α, β, and γ-cyclodextrins. Preparation of cycloamylose sulfates and sulfonates, and modified cyclodextrin sulfates and sulfonates are described in the art. See, for example, U.S. Pat. Nos. 2,923,704; 4,020,160; 4,247,535; 4,258,180; 4,596,795 and 4,727,064, each of which is hereby incorporated by reference. These cyclodextrin sulfates and sulfonates are typically associated with a physiologically acceptable cation.

According to another embodiment, the hydroxyl groups are substituted with alkyl ether sulfonates of the formula -O-(C_1-C_8 alkyl)-SO_3. In one example, commercially available Captisol® (Cydex) may be used which is a sulfobutyl ether derivative of β-cyclodextrin having an average of seven sulfobutyl ether groups per cyclodextrin molecule (*i.e.*, O-R-Y is -O-$(CH_2)_4$-$SO_3^-Na^+$). Captisol does not exhibit the nephrotoxicity associated with underivitized β-cyclodextrin. Additional cyclodextrin derivatives are disclosed in U.S. Patent Nos. 5,134,127; 6,165,995 and 6,060,597, each of which is hereby incorporated by reference.

The nephrotoxic drug may be any pharmaceutical agent including small molecules and peptides that cause renal damage upon administration to a host. Such drugs include, by way of example, diuretics, NSAIDs, ACE inhibitors, cyclosporin, tacrolimus, radiocontrast media, interleukin-2, vasodilators (hydralazine, calcium-channel blockers, minoxidil, diazoxide), mitomycin C, conjugated estrogens, quinine, 5-fluorouracil, ticlopidine, clopidogrel, interferon, valacyclovir, gemcitabine, bleomycin, heparin, warfarin, streptokinase, aminoglycosides, cisplatin, nedaplatin, methoxyflurane, tetracycline, amphotericin B, cephaloridine, streptozocin, tacrolimus, carbamazepine, mithramycin, quinolones, foscarnet, pentamidine, intravenous gammaglobulin, fosfamide, zoledronate, cidofovir, adefovir, tenofovir, mannitol, dextran, hydroxyethylstarch, lovastatin, ethanol, codeine, barbiturates, diazepam, quinine, quinidine, sulfonamides, hydralazine, triamterene, nitrofurantoin, mephenytoin, penicillin, methicillin ampicillin, rifampin, sulfonamides, thiazides,

cimetidine, phenytoin, allopurinol, cephalosporins, cytosine arabinoside, furosemide, interferon, ciprofloxacin, clarithromycin, telithromycin, rofecoxib, pantoprazole, omeprazole, atazanavir, gold, penicillamine, captopril, lithium, mefenamate, fenoprofen, mercury, interferon, pamidronate, fenclofenac, tolmetin, foscarnet, aciclovir, methotrexate, sulfanilamide, triamterene, indinavir, foscarnet, ganciclovir, methysergide, ergotamine, dihydroergotamine, methyldopa, pindolol, hydralazine, atenolol, taxol, tumor necrosis factor, chlorambucil, interleukins, bleomycin, etoposide, fluorouracil, vinblastine, doxorubicin, cisplatin and the like (see, generally, Devasmita et al., *Nature Clinical Practice Nephrology* (2006) **2**, 80-91).

Methotrexate

According to one embodiment, the nephrotoxic drug is methotrexate, or a derivative or pharmaceutically acceptable salt thereof. Methotrexate (N-[4-[[(2,4-diamino-6pteridinyl)methyl]methylamino]benzoyl]-L-glutamicacid) is an S-phase chemotherapeutic antimetabolite used for the treatment of various neoplasms, particularly CNS lymphoma. MTX is one the most widely used anticancer agents and is employed in the treatment of neoplastic diseases such as gestational choriocarcinoma, osteosarcoma, chorioadenoma destruens, hydatidiform mole, acute lymphocytic leukemia, breast cancer, epidermoid cancers of the head and neck, advanced mycosis fungoides, lung cancer, and non-Hodgkins lymphomas (Physicians Desk Reference (45th ed.), Medical Economical Co., Inc., 1185-89 (Des Moines, Iowa (1991))). MTX is also an effective immunosuppressive agent, with utility in the prevention of the graft-versus-host reaction that can result from tissue transplants, as well as in the management of inflammatory diseases. Consequently, MTX can be employed in the treatment of severe and disabling psoriasis and rheumatoid arthritis (Hoffmeister, *The American Journal of Medicine* (1983) 30:69-73 ; Jaffe, *Arthritis and Rheumatism* (1988) 31: 299).

However, methotrexate is associated with renal and hepatic toxicity when applied in the "high dose regimen" that is typically required for maximum efficiency (Barak et al., *J. American Coll. Nutr.* (1984) 3:93-96).

Numerous patents disclose MTX and MTX analogs, any of which may be used in practicing the present invention. See, for example, U.S. Pat. Nos. 2,512,572, 3,892,801, 3,989,703, 4,057,548, 4,067,867, 4,079,056, 4,080,325, 4,136,101, 4,224,446, 4,306,064, 4,374,987, 4,421,913, 4,767,859, 3,981,983, 4,043,759,

4,093,607, 4,279,992, 4,376,767, 4,401,592, 4,489,065, 4,622,218, 4,625,014, 4,638,045, 4,671,958, 4,699,784, 4,785,080, 4,816,395, 4,886,780, 4,918,165, 4,925,662, 4,939,240, 4,983,586, 4,997,913, 5,024,998, 5,028,697, 5,030,719, 5,057,313, 5,059,413, 5,082,928, 5,106,950, 5,108,987, 4,106,488, 4,558,690, 4,662,359, 6,559,149, each of which is hereby incorporated by reference. Other MTX analogs and related antifolate compounds include trimetrexate, edatrexate, AG331, piritrexim, 1843U89, LY 231514, ZD 9331, raltritrexed, lometrexol, MTA and AG337 (Takimoto, *Seminars in Oncology* (1997) 24:S18-40-51; Sorbello et al., *Haematoligica* (2001) 86:121-27); CB 3717, LY 309887 (Calvert, *Seminars in Oncology* (1999) 26:S6, 3-10; Rosowsky, *Progress in Med.Chem.* (1989) 26:1-237)).

Accordingly, the compositions disclosed herein may be used for treating cancer or for inhibiting the growth of cancer; as well as for treating multiple sclerosis, and the symptoms associated therewith. The compositions may be used in conjunction with or in combination with other active agents such as interferon. Additionally, the compositions may be used for treating autoimmune disorders such as lupus and rheumatoid arthritis.

Antibiotics

Aminoglycoside antibiotics, such as gentamicins, kanamycins, streptomycins and tobramycins, are generally utilized as broad spectrum antimicrobials effective against, for example, gram-positive, gram-negative and acid-fast bacteria. However, aminoglycosides are often associated with undesired side-effects such as nephrotoxicity and ototoxicity. Other antibiotics which may used in the practice of the present invention include vancomicin and cephalosporin, such as Rocephin® and Kefzol.

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs all present renal toxicity. NSAIDs typically are used to reduce pain while avoiding the use of opiate derivatives. Two widely used NSAIDs are Indomethacin and Toradol® manufactured by Roche Pharmaceuticals.

Antifungal Agents

Caspofungin and amphotericin B are both known to be nephrotoxic, and may be used in practicing the disclosed invention.

Anti-cancer agents

Many anti-cancer agents exhibit dose limiting renal toxicity and may be used in practicing the present invention. Such agents include, by way of example, cisplatin, doxyrubicin, cyclophosphamide, butasulfane, and the like.

Contrast Agents

Contrast agents are injected into a patient prior to x-ray scans. Contrast agents are highly concentrated (50-66% solutions) iodinated compounds. In view of this high concentration it is likely that only a maximum of a 1:1 ratio of contrast agent to cyclodextrin would be necessary. Examples of the possible use of cyclodextrin to protect against renal damage by contrast agents include Iohexol and Ioversol. Other contrast agents that may be used in practicing the present invention include diatrizoate meglumine and ioxaglate.

Administration

The oligosaccharide may be complexed with the nephrotoxic drug, although it is not believed necessary for antinephrotoxic protective effect of the composition. The ratio of drug to oligosaccharide is preferably within a range such that the drug does not precipitate at pH typically found in the kidney, taking into account the transit time of the drug through the kidney. In some cases, it may be desirable to minimize the amount of oligosaccharide *in vivo*. Simple *in vitro* solubility experiments such as described in the Examples may be used to determine the minimum amount of oligosaccharide needed to effectively protect from renal damage. In one embodiment, the molar ratio of drug : oligosaccharide is greater than 1:1, and may range from about 1.1:1 to about 50:1, preferably from about 1.25:1 to about 25:1; more preferably from about 1.75:1 to about 2:1 to about 10:1. In the case of methotrexate, by way of example only, it was found that a mole ratio of about 2:1 methotrexate:Captisol worked well to keep methotrexate in solution *in vitro* and provided the desired nephrotoxic effect. Where lower amounts of oligosaccharides are desired, it is contemplated that additional solubilizing agents may be used, provided that the amount of oligosaccharide in the composition remains sufficient to provide a renal protective effect.

It may be desirable in some cases to have a ratio of drug to oligosaccharide lower than 1:1 where, for example, the binding constant of the drug is low or where the drug is processed by the kidneys at a lower rate than the cyclodextrin, it may be beneficial to have a molar excess of oligosaccharide. This may be true for many

classes of drugs where the dose of the drug required for a therapeutic effect is low, therefore, although the molar ratio of oligosaccharide might be higher, the absolute amount or concentration *in vivo* is not necessarily increased. As such, the composition may comprise from about 2 to about 50; from about 2 to about 20; or from about 2 to about 10 times molar excess of the oligosaccharide, or preferably in the range of from about 1 to about 5:1, and more preferably in the range of about 2 to about 5:1, oligosaccharide to drug.

Further provided are methods of reducing the nephrotoxic effect of a pharmaceutically active compound comprising contacting the compound with a polyanionic oligosaccharide. Methods are included for inhibiting or reducing nephrotoxicity associated with a nephrotoxic drug, comprising administering a pharmaceutical composition comprising a polyanionic oligosaccharide, the nephrotoxic drug and optionally a pharmaceutically acceptable carrier. Although it is preferred that administration occur as a single dose, particularly where the oligosaccharide assists in solubolizing the drug, the methods may also be effected by concurrently administering a pharmaceutical composition comprising a polyanionic oligosaccharide and a pharmaceutical composition comprising the nephrotoxic inducing drug, *i.e.*, in separate doses. Where the drug and oligosaccharide are combined into a single dosage unit, they may be combined with a pharmaceutically acceptable carrier, for example, a cosolution or dispersion in an inert pharmaceutically acceptable solvent or dispersing agent or the like.

Alternatively, the oligosaccharide can be separately formulated with pharmaceutically acceptable materials and administered separately; either concurrently with the drug or within about an hour before or after administration of the drug. By concurrently, it is meant administration of the separate doses occurs substantially at the same time such that both the oligosaccharide and the drug are present *in vivo*. Alternatively, administration may occur sequentially provided that the oligosaccharides are present within the renal environment as the concentration of drug in the kidney increases to levels at which the toxic effect of the drug may occur.

The mode of administration, the dosage and frequency of dosage is governed by the mode of administration and dosage considerations conventionally employed with the pharmaceutical agent. Thus, for example, various combinations of the invention can be administered intramuscularly or intravenously, or otherwise, as dictated by medical and pharmacological practice related to the desired use of the

particular drug or agent employed. Administration may be achieved orally or parenterally including, *inter alia*, topical application, intravenous, intra-arterial or subcutaneous injection, and including absorption as well as injection and introduction into body apertures or orifices.

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

The other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Examples

Example 1. Effect of pH on Solubility of MTX

Solubility studies were performed to determine whether Captisol could prevent the precipitation of MTX over a time period greater than transit time of MTX through the kidney (i.e., less than 2 minutes). Solutions were prepared as shown in the Table 1. Each solution was acidified with HCl, centrifuged and aliquots of the supernatant removed as a function of time, and the concentration of MTX in solution was measured spectrophometrically.

As shown in Figure 1, Captisol prevented the precipitation of MTX at a concentration dependent rate. At a mole ratio of 1:1, the MTX remains in solution indefinitely. At lower ratios, precipitation occurs at a concentration dependent rate. At 0.50:1 ratio of Captisol to MTX, MTX remains in solution for at least 15 minutes, and at a ratio of 0.25:1, much of it remains for 10 minutes. In view of the rapid transit time of filtration in the kidneys, *in vivo* experiments to determine the optimal ratio of MTX to Captisol to prevent kidney damage may be performed.

Table 1.

MTX-captisol solubility
MTX added: 0.91 mg/ml
Concentration of MTX: 0.002 M
Rate of precipitation of MTX at pH 5.0 in presence and absence of captisol
Captisol:MTX molar ratio 1:1

Capti sol	Time after	OD at	Amount of MTX	correction for dilution		conc. of MTX	captisol/ MTX

adde d			in solution				ratio
(mM)	adding acid	302-304nm	1:25 dilution				
	(min)		(ug/mL)	ug/ml	mg/ml	(M)	
0.002	5	2.71	33.28800989	832.2002	0.8322	0.0018308	1:1
0.002	10	2.291	28.10877627	702.7194	0.702719	0.001546	1:1
0.002	15	2.296	28.17058096	704.2645	0.704265	0.0015494	1:1
0.002	20	2.127	26.0815822	652.0396	0.65204	0.0014345	1:1
0.002	30	2.067	25.33992583	633.4981	0.633498	0.0013937	1:1
0.002	40	2.202	27.00865266	675.2163	0.675216	0.0014855	1:1
0.002	50	2.078	25.47589617	636.8974	0.636897	0.0014012	1:1
0.002	60	2.004	24.56118665	614.0297	0.61403	0.0013509	1:1

Captisol:MTX molar ratio 0.5:1

Captis ol added	Time after adding acid	OD at 302-304nm	MTX in solution	correction for dilution1:500 1:25 dilution		conc. of mtx	captisol/MTX ratio
(M)	(min)	nm	(ug/mL)	ug/ml	mg/ml	(M)	
0.001	5	1.854	22.70704574	567.6761	0.567676	0.0012489	**0.5:1**
0.001	10	2.263	27.76266996	694.0667	0.694067	0.0015269	**0.5:1**
0.001	15	2.594	31.85414091	796.3535	0.796354	0.001752	**0.5:1**
0.001	20	1.531	18.7144623	467.8616	0.467862	0.0010293	**0.5:1**
0.001	30	1.044	12.6946848	317.3671	0.317367	0.0006982	**0.5:1**
0.001	40	0.959	11.64400494	291.1001	0.2911	0.0006404	**0.5:1**
0.001	50	0.864	10.4697157	261.7429	0.261743	0.0005758	**0.5:1**
0.001	60	0.861	10.43263288	260.8158	0.260816	0.0005738	**0.5:1**

Captisol:MTX molar ratio 0.25:1

Captiso l added	Time of OD	OD at 302-304nm	conc of MTX	dilution1:50 0		molarity of mtx	captisol/ MTX ratio
(M)	min after addin g acid	nm	(ug/mL)	ug/ml	mg/ml	(M)	
0.0005	5	2.363	28.99876391	724.9691	0.724969	0.0015949	**0.25:1**
0.0005	10	1.798	22.01483313	550.3708	0.550371	0.0012108	**0.25:1**
0.0005	15	1.173	14.28924598	357.2311	0.357231	0.0007859	**0.25:1**
0.0005	20	0.864	10.4697157	261.7429	0.261743	0.0005758	**0.25:1**
0.0005	30	0.686	8.26946848	206.7367	0.206737	0.0004548	**0.25:1**
0.0005	40	0.633	7.61433869	190.3585	0.190358	0.0004188	**0.25:1**
0.0005	50	0.556	6.662546354	166.5637	0.166564	0.0003664	**0.25:1**
0.0005	60	0.556	6.662546354	166.5637	0.166564	0.0003664	**0.25:1**

Captisol:MTX molar ratio 0:1

Captiso l added	Time of OD	OD at 302-304nm	conc of MTX	dilution1:50 0		molarity of mtx	captis ol/MT X ratio
(M)	min after addin g acid	nm	(ug/mL)	ug/ml	mg/ml	(M)	
0	5	0.296	3.448702101	86.21755	0.086218	0.0001897	**0:1**
0	10	0.22	2.509270705	62.73177	0.062732	0.000138	**0:1**
0	15	0.274	3.176761434	79.41904	0.079419	0.0001747	**0:1**
0	20	0.328	3.844252163	96.1063	0.096106	0.0002114	**0:1**
0	30	0.325	3.807169345	95.17923	0.095179	0.0002094	**0:1**
0	40	0.284	3.300370828	82.50927	0.082509	0.0001815	**0:1**
0	50	0.293	3.411619283	85.29048	0.08529	0.0001876	**0:1**
0	60	0.333	3.90605686	97.65142	0.097651	0.0002148	**0:1**

Example 2. Protective Effect of Captisol - MTX

A comparison of the effect of MTX 40 mg/kg with and without Captisol® (molar ratio 1:1) in myelin-oligodendrocyte-glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE) in C57BL6 mice was performed. Clinical signs, CNS pathology and renal pathology were measured.

EAE was induced as follows. Mice were anesthetized with Avertin (222 tribromoethanol) and given two subcutaneous injections of 150 μg of MOG in PBS (total dose 300 μg) that had been emulsified in an equal volume of Freund's incomplete adjuvant containing 250 μg of M. tuberculosis $H_{37}RA$ (total dose 500 μg). One injection was given at the nape and the second was given on the dorsum. Pertussis toxin (100 ng; i.v. through the tail vein) was administered on days 0, 3, and 7 following encephalitogen.

Stock MTX was used at 25 mg/ml (Bedford laboratories). MTX stock was diluted 3.67 times with PBS (2 ml stock + 5.34 ml PBS) for a total volume of 7.34 ml, at a concentration of 6.8 mg/ml (14.9 mM).

3.00 ml of diluted MTX solution was aliquoted and added to 96.6 mg of Captisol powder, and the solution vortex mixed. The resulting solution was mostly clear but pale yellow. All solutions were kept at RT in dark until ready to inject. 96.6 mg/ 3 ml = 32.228 mg/ml = 14.9 mM; MTX: Captisol molar ratio 1:1.

The test mixtures were administered to 5 groups of mice (Groups I - V; see Table 2).

Table 2.

Groups	**EAE/control**	**Treatment**	**Number of mice**
Group I	EAE	PBS	5
Group II	EAE	MTX 40 mg/kg	10
Group III	EAE	MTX 40 mg/kg +captisol (molar ratio 1:1)	10
Group IV	EAE	Captisol only 201.25 mg/kg	10

Group V	EAE	No treatment	5

Twenty four (24) hours after symptom onset, MTX (40 mg/kg body weight) was administered via tail vein. Injection volume were between 100 -120 µl. MTX + Captisol was injected in a volume of 100-120 µl via tail vein. Captisol alone (32.22 mg/ml) was injected via tail vein in a volume of 100-120µl. Leukovorin (20 mg/kg body weight) was injected via tail vein 4 hours after MTX injection and again 24 hours after. Leukovorin, the active metabolite of folic acid, is typically given with methotrexate in anti-cancer chemotherapy to help protect normal cells.

Animals were weighed and scored daily for clinical signs. Scores were based on the following on the following signs:

0-normal

1-flacid tail, piloerection, and /or weight loss

2-hind limb weakness righting difficulty

3-hind limb weakness causing righting inability

4-hindlimb paresis, limp walking, and or/incontinence

5-partial hind limb paralysis

6- total hind limb paralysis plus forelimb weakness

7- total hind limb paralysis plus forelimb paresis or paralysis

8-death or moribund requiring sacrifice

Mice were scored daily for disease severity and then sacrificed on day 10 of disease. Brain, kidney and spleen were formalin fixed. To assess the infiltration of T cells into the CNS, CD3+ immunohistochemistry was performed on paraffin embedded 8 micron thick hind brain sections of untreated EAE mice and EAE mice treated concurrently with MTX and captisol. For light microscopic investigation, kidneys were fixed in 10% buffered formalin and processed routinely for paraffin embedding. Tissue sections of 5 micron meter were stained with hematoxylin and eosine and examined under Nikon coolpix light microscope.

The severity of total kidney damage was evaluated by a semiquantitative measurement of damage as described. Each tissue section of the kidney was assessed for degeneration of glomerular structure, glomerular crowding and congestion, dilation of bowman space, degeneration of proximal and distal tubules, and dilation of renal tubules, vascular congestion, and inflammatory cell infiltration. For glomerular

atrophy, glomeruli that contained more than 20 nuclei were scored "0", and those containing less than 10 nuclei were scored "4." Intermediate stages were 1, 2 and 3. Other criterion was scored on a 0-3 scale: 0=none; 1=mild; 2=moderate; 3=severe.

The microscopic score of each tissue was calculated as the sum of the scores given to each criterion and at least 100 nephrons (glomeruli plus surrounding tubules) were analyzed per section. Data is represented as mean ± SEM (see Figure 2).

MOG treated mice developed severe clinical manifestation, starting on days 10-11. They were maintained until sacrifice at 13 days. All animal were affected. Partial or complete hind leg paralysis (clinical score).

Efficacy of MTX 40 mg/kg + Captisol was comparable to the efficacy seen with MTX treatment alone.

The result of the CNS Pathology – $CD3^+$ Immunostaining showed that untreated EAE mice had extensive infiltration of $CD3^+$ T cells in the hind brain and spinal cord (not shown). EAE mice treated post-symptomatically with concurrently MTX+Captisol (40 mg/kg + captisol 1:1 molar ratio) showed 80-90% reduction in T cell infiltration (not shown). The efficacy of MTX+Captisol was comparable to the efficacy with MTX treatment alone.

Renal pathology scores are shown in Figure 3. Kidney sections from 3 different EAE mice treated with MTX 40 mg/kg, showed dilation of renal tubules and degeneration of proximal tubules. Kidney sections from mice treated concurrently with MTX+Captisol showed a protective effect on tubules. A single intravenous bolus injection of 40 mg/kg MTX produced morphological changes in the kidney which was mostly restricted to dilation of renal tubules in the cortex. Concurrent Captisol administration with MTX resulted in reduction in pathology score in the EAE mice.

Example 3. Captisol - MTX at Various Molar Ratios.

A study of histopathological changes in the kidney after single bolus intravenous MTX with or without concurrent captisol at different molar ratios was performed.

MTX solutions were prepared as follows. Solution A (24 mg/ml (53 mM)) was prepared from stock solution of 25 mg/ml (Methotrexate from Bedford laboratories) in sterile PBS (total volume 6 mL). Solution B was prepared by diluting 3 ml of Solution A to 1:1.33 in PBS to obtain a working dilution of 18 mg/mL (39.6

mM), pH 7.4. Solution C was prepared by diluting 2 mL of solution A 1:2 to obtain a working dilution of 12 mg/mL (26.4 mM), pH 7.2.

MTX + Captisol solutions were prepared as follows. Solution D was made by adding 57.32 mg Captisol to an aliquot of 500 micro liter of solution A (molar ratio 1:1, neutral pH). Solution E was made by adding 85.6 mg Captisol to 1 mL aliquot of solution B (molar ratio 1:1, neutral pH). Solution F was made by adding 57.10 mg Captisol to 1 mL aliquot of solution C (molar ratio 1:1, neutral pH). Solution G was prepared by adding 42.8 mg Captisol to 1 mL aliquot of solution B. Solution H was made by adding 28.6 mg of Captisol to 1 mL aliquot of solution C.

MTX and Captisol were administered to 8 groups of mice (Groups I-VIII; see Table 3) via tail vein by a single injection in a volume of 100 - 120μL. Animals were sacrificed after 48 hours. Clinical symptoms, body weights were recorded. Kidneys were preserved (one frozen, one formalin fixed) for pathology. Leukovorin was given at 4 hours and after 18 hours. Urine was not alkalinized.

Table 3.

GROUPS		TREATMENT	Molarity of MTX	Molarity of Captisol	Molar ratio MTX:Captisol	# of mice
Group I	Normal mice	MTX only, 80 mg/kg, iv bolus. Solution A	26.4 mM			3
Group II	Normal mice	MTX only, 120 mg/kg, iv, bolus. Solution B	39.6 mM			3
Group III	Normal mice	MTX only, 160 mg/kg, iv, bolus. Solution C	53 mM			1
Group IV	Normal mice	MTX, 80 mg/kg, iv + Captisol 382 mg/kg body weight. Solution D	26.4 mM	26.4 mM	1:1	3
Group V	Normal mice	MTX, 120 mg/kg, iv + Captisol 570 mg/kg. Solution E	39.6 mM	39.6 mM	1:1	3
Group VI	Normal mice	MTX, 160 mg/kg, iv + Captisol 764 mg/kg body weight. Solution F	53 mM	53 mM	1:1	1
Group VII	Normal mice	MTX, 80 mg/kg, iv + Captisol 191 mg/kg. Solution G	26.4 mM	13.2 mM	1:0.5	3

Group VIII	Normal mice	MTX, 120 mg/kg, iv + Captisol 285 mg/kg. Solution H	39.6 mM	19.8 mM	1:0.5	3

Semi quantitative estimates of total kidney damage in mice treated with MTX 80 mg/kg, 120 mg/kg, and a combination of MTX + Captisol at MTX to Captisol molar ratio of 1:1 and 1:0.5, are shown in Figure 4. The scores for the control kidney were between 3-4 (not shown in the graph). Hematoxylin and eosin sections of paraffin embedded kidney were analyzed microscopically for extent of renal damage. Data is expressed as mean $\pm$ SEM. Data for MTX 160 mg/kg is not included in the graph as there was only a single mouse in this group.

Paraffin sections of the kidneys stained with hematoxylin and eosin (not shown) showed that kidneys from mice administered 80 mg/kg MTX had dilation of tubules, degenerative changes in the tubules, hypercellularity in the glomerulus and atypical collection of interstitial cells and convulated tubules. Kidneys from mice administered 80 mg/kg MTX + Captisol at MTX to Captisol molar ratio 1:1, and 80 mg/kg MTX + Captisol at MTX to Captisol molar ratio 1:0.5, showed milder degeneration of tubules, normal glomerulus, and no infiltration of inflammatory cell. However, the degree of kidney protection was much higher when the molar ratio of MTX to Captisol was 1:0.5.

Paraffin sections of kidney stained with hematoxylin and eosin (not shown) showed that kidneys from mice administered 120 mg/kg MTX, IV had glomerular atrophy, degeneration of basement membrane on bowman capsule, degenerative changes in the tubules, hypercellularity in the glomerulus and infiltration of mononuclear cells. Kidneys from mice administered 120 mg/kg MTX + captisol at MTX to Captisol molar ratio of 1:1, and 120 mg/kg MTX + captisol at MTX to Captisol molar ratio of 1:0.5, showed mostly normal glomerulus, protection of proximal and distal tubules and no infiltration of inflammatory cells.

Example 4. Captisol - MTX Time Studies

MTX - Captisol mixtures were administered as set forth in Table 4.

Table 4.

Normal Mice	**Treatment** Single intravenous bolus injection via tail vein	**Tissue collection**	**MTX:Captisol molar ratio**	**Number of mice**
	MTX 160 mg/kg	kidney collected at 24 hrs after drug administration	1:0	4
	MTX 160 mg/kg+ captisol 764 mg/kg		1:1	4
	MTX 160 mg/kg+ captisol 382 mg/kg		1:0.5	4
	MTX 160 mg/kg+captisol 191 mg/kg		1:0.25	4
Normal Mice	MTX 160 mg/kg	kidney collected at 48 hrs after drug administration	1:0	4
	MTX 160 mg/kg+ captisol 764 mg/kg		1:1	4
	MTX 160 mg/kg+ captisol 382 mg/kg		1:0.5	4
	MTX 160 mg/kg+captisol 191 mg/kg		1:0.25	4
Normal Mice	MTX 160 mg/kg	Kidney collected after 1 week after drug administration	1:1	4
	MTX 160 mg/kg+ captisol 764 mg/kg			4

For light microscopic investigation, kidneys were fixed in 10% buffered formalin and processed routinely for paraffin embedding. Tissue sections of 5 micron

meter were stained with hematoxylin and eosine and examined under a Nikon coolpix light microscope, photography was done with a Nicon Coolpix camera. The severity of total kidney damage was evaluated by a semiquantitative measurement of damage as described below. Each tissue section of the kidney was assessed for degeneration of glomerular structure, glomerular crowding and congestion, dilation of bowman space, degeneration of proximal and distal tubules, and dilation of renal tubules, vascular congestion, and inflammatory cell infiltration. For glomerular atrophy, glomeruli that contained more than 20 nuclei were scored "0", and those containing less than 10 nuclei were scored "4." Intermediate stages were 1, 2 and 3. Other criterion was scored on a 0-3 scale: 0=none; 1=mild; 2=moderate; and 3=severe.

The microscopic score of each tissue was calculated as the sum of the scores given to each criterion and at least 100 nephrons (glomeruli plus surrounding tubules) were analyzed per section. See, e.g., Bhat et al., *PNAS* (2003) 100(7); Sener et al., *Cell Biol Toxicol* (2006) 22:470-60.

Referring to Figure 5, kidney tissue of MTX treated mice shows extensive histopathological changes after 24 and 48 hours. At 24 hours and 48 hours there was atrophy of the glomerulus, degeneration and dilation of Bowmans space, and inflammatory cell infiltration in the interstitium and tubular degeneration. The Captisol + MTX treated group showed milder glomerular and tubular changes and less infiltration of inflammatory cells. However, some of the changes seen after 24 hours appeared to be reversible since the cumulative pathological score was less at 48 hours compared to 24 hours.

Histopathological changes in the kidney were studied at 24 hours post MTX injection with or without captisol coadministration. Mice were sacrificed after 24 hours following MTX or MTX + Captisol coadministration. Paraffin sections of kidney were stained with hematoxylin and eosin (not shown). MTX 160 mg/kg administration resulted in degeneration of glomerular structure and dilation of Bowman's space, degeneration of proximal and distal tubules and inflammatory cell infiltration. Milder glomerular and tubular denegation was observed for MTX 160 mg/kg + Captisol at MTX to Captisol molar ratio of 1:1. MTX 160 mg/kg + Captisol at MTX to Captisol molar ratio of 1:0.5 were most effective in the preservation of glomerular and tubular structures. Infiltration of inflammatory cells was completely absent. MTX 160 mg/kg + Captisol used in molar ratio of 1:0.25 did not protect against kidney damage.

Histopathological changes were recorded for the kidney at 48 hours post MTX injection with or without Captisol coadministration. Mice were sacrificed after 48 hours following MTX or MTX + Captisol administration. Paraffin section of kidney were stained with hematoxylin and eosin (not shown). At 48 hours post injection, MTX 160 mg/kg administration (i.v.) resulted in degeneration of glomerular structure, dilation of Bowman's space, dilation of proximal and distal tubules, degeneration of proximal and distal tubules and inflammatory cell infiltration. Milder glomerular and tubular denegation was observed for MTX 160 mg/kg + Captisol (MTX to Captisol molar ratio of 1:1). MTX 160 mg/kg + Captisol in 1:0.5 molar ratio resulted in relatively better preservation of glomerular and tubular structure. Infiltration of inflammatory cells was completely absent. MTX 160 mg/kg + Captisol used in molar ratio of 1:0.25 were not effective in protecting the kidney from MTX induced damage. The greatest protection was seen when MTX to Captisol molar ratio was 1:0.5.

Histopathological changes were also studied for the kidney after 1 week post MTX administration. Mice were sacrificed after 1 week following MTX 160 mg/kg, single i.v. bolus injection. Paraffin sections of kidney were stained with hematoxylin and eosin (not shown). MTX administration alone resulted mainly in degeneration of proximal and distal tubules with occasional glomerular atrophy or crowding. Degenerating cells with swollen nuclei were seen lining the proximal and distal tubules. Occasionally some tubules were seen to be lined by double cell layer. Some tubules were congested with eosinophilic materials. The pathological changes were mostly found in the cortical areas of the kidney. Most of the glomerular structures were normal.

Histopathological changes were recorded for the kidney after 1 week post MTX + Captisol injection. Mice were sacrificed after 1 week following MTX + Captisol coadministration. Paraffin sections of kidney were stained with hematoxylin and eosin from four separate mice (not shown). Kidneys of mice coadministered with MTX 160 mg/kg + Captisol at MTX to Captisol molar ratio of 1:1 showed significantly less glomerular disruption and greater preservation of the tubules. Infiltration of inflammatory cells was absent.

Example 5. Evaluation of the Nephroprotective Effect of Captisol in Doxorubicin Induced Nephrotoxicity Model in Mice.

Female C57BL/6 mice were injected intravenously with a single dose of (10 mg/kg) doxorubicin. The mice were sacrificed after 72 hours. The development of glumerular and tubulointerstitial injury after doxorubicin and doxorubicin + Captisol was evaluated by means of renal histology. Paraffin sections of 5 μM were cut and stained with H&E and periodic acid Schiff (PAS). They were examined by light microscopy and scored in a blinded fashion. Thirty glomeruli and neighboring tubules were scored at superficial cortes (near the surface of the capsule). One hundred glomeruli and neighboring tubules were scored at the level of deep renal cortex and around the outer strips of the outer medulla.

Figure 6 shows the mean pathology scores for each treatment group at the level of superficial renal cortex. Figure 7 shows the renal pathology scores for individual mice treated with doxorubicin or doxorubicin + Captisol at the level of superficial renal cortex. Figure 8 shows the mean pathology scores for each treatment group at the level of deep renal cortex + outer medulla. Figure 9 shows the renal pathology scores for individual mice treated with doxorubicin or doxorubicin + Captisol at the level of deep renal cortex + outer medulla.

None of the control or Captisol treated mice had any tubulointerstitial changes. The doxorubicin treated group showed tubular casts, abundant dilated tubules and moderate loss of brush border in the some proximal tubules. Some of the glomeruli were collapsed and at various stages of degeneration. The pathology was found to be more prominent in the outer periphery of the renal cortex. Tubular atrophy or neutrophils infiltration was not seen. In doxorubicin + captisol treated mice there was almost 71% reduction of degeneration and almost 72% reduction in tubular dilatation. At deeper renal cortex and medulla, there was a 90% reduction in glomerular degeneration and a 50% reduction in tubular dilatation.

Example 6. Evaluation of the Nephroprotective Effect of Captisol in Cisplatin Induced Nephrotoxicity Model in Mice

Female C57BL/6 mice were injected intravenously with a single dose of (10 mg/kg) cisplatin (N = 5) or cisplatin + Captisol at cisplatin to Captisol molar ratio of 1:1, 1:0.5 and 1:0.25 (N = 5, N = 4 and N = 6, respectively). The animals were sacrificed after 72 hours.

The development of glumerular and tubulointerstitial injury after cisplatin and protection by cisplatin + Captisol was evaluated by means of renal histology. Paraffin sections of 5 μM were cut and stained with H&E and periodic acid Schiff (PAS). They were examined by light microscopy and scored in a blinded fashion. Thirty glomeruli and neighboring tubules were scored at the superficial cortex (near the surface of the capsule). One hundred glomeruli and neighboring tubules were scored at the level of deep renal cortex and around the outer strips of the outer medulla.

Figure 10 shows the mean scores at the level of the superficial cortex in cisplatin and cisplatin + Captisol treated groups (cisplatin to Captisol molar ratio 1:1, 1:0.5 and 1:0.25). Figure 11 shows the pathology scores of individual mice in each treatment group at the level of the superficial renal cortex. Figure 12 shows the mean scores at the level of the deep cortex and outer medulla in cisplatin and cisplatin + Captisol treated groups (cisplatin to Captisol molar ratio 1:1, 1:0.5 and 1:0.25). Figure 13 shows pathology scores of individual mice in each treatment group at the level of the deep renal cortex and outer medulla.

None of the control mice had any tubulointerstitial changes. The cisplatin treated mice showed necrosis, sloughing of tubular epithelial cells and loss of brush border in the some proximal tubules. Abundant presence of dilated tubules was a prominent feature of cisplatin induced nephrotoxicity. Some of the glomeruli were collapsed and some showed early degenerative changes. All of these changes were significantly less pronounced by treatment with cisplatin + Captisol, demonstrating that Captisol protected the kidney at both cisplatin:Captisol 1:1 and cisplatin:Captisol 1:0.5.

1. A pharmaceutical composition comprising a substituted cyclodextrin, a nephrotoxic anticancer drug and a pharmaceutically acceptable carrier, said cyclodextrin being present in an amount effective for substantially inhibiting the nephrotoxic effect of said drug, and wherein the molar ratio of drug:cyclodextrin is greater than 1:1.

2. The composition of claim 1 wherein said cyclodextrin is substituted with one or more charged moieties.

3. The composition of claim 2 wherein said moieties are selected from the group consisting of, sulfonate, sulfate, carboxylate, phosphonate and phosphate groups.

4. The composition of claim 1 wherein said cyclodextrin is of the formula:

- cyclodextrin-$[(O\text{--}R\text{--}Y)^{-}\ (Me)^{+}]_n$

where R is selected from the group consisting of straight-chained or branched $C_{1\text{-}10}$ alkyl, alkenyl or alkynyl; $C_{3\text{-}8}$ cycloalkyl and $C_{3\text{-}8}$ aryl, each ring optionally containing 1 or more heteroatoms selected from S, N and O; and optionally substituted with halo or hydroxyl;
Y is an anionic group selected from the group consisting of COO, SO_4, SO_3, PO_3H and PO_4;

Me is a pharmaceutically acceptable cation; and

n is a whole number greater than 1.

5. The composition of claim 4 wherein R is $C_{1\text{-}10}$ alkyl, optionally substituted with halo or hydroxyl.

6. The composition of claim 5 wherein said cyclodextrin is selected from the group consisting of alpha, beta or gamma cyclodextrin.

7. The composition of claim 1 wherein the molar ratio of drug : cylodextrin is at least about 2:1.

8. The composition of claim 1 wherein said drug is a positively charged pharmaceutical agent.

9. Use of a pharmaceutically active anticancer compound that has a nephrotoxic effect, a substituted cyclodextrin and a pharmaceutically acceptable carrier in the manufacture of a medicament for reducing the nephrotoxic effect of said pharmaceutically anticancer active compound, said cyclodextrin being present in an amount effective for substantially reducing said nephrotoxic effect of said pharmaceutically active compound, and wherein the molar ratio of anticancer drug:cyclodextrin is greater than 1:1.

10. Use of a substituted cyclodextrin in the manufacture of a first pharmaceutical composition for inhibiting nephrotoxicity associated with administration of a nephrotoxic inducing anticancer drug, wherein said first pharmaceutical composition is to be concurrently administered with a second pharmaceutical composition comprising the nephrotoxic inducing anticancer drug, and wherein the molar ratio of drug:cyclodextrin is greater than 1:1.

11. The composition of claim 4 wherein said drug is methotrexate or a derivative or pharmaceutically acceptable salt thereof.

12. The composition of claim 11 wherein said cyclodextrin is beta-cyclodextrin; (O--R--Y)$^{-}$(Me)$^{+}$ is --O--$(CH_2)_4$--SO_3-Na^{+}, and n is about 7.

13. Use of a therapeutically effective amount of the composition of claim 11 in the manufacture of a medicament for treating the symptoms associated with multiple sclerosis in a patient in need thereof.

14. Use of a therapeutically effective amount of the composition of claim 11 in the manufacture of a medicament for treating cancer in a host in need thereof.

15. Use of a therapeutically effective amount of the composition of claim 11 in the manufacture of a medicament for inhibiting the growth of cancer in a host in need thereof.

16. Use of a therapeutically effective amount of the composition of claim 11 in the manufacture of a medicament for treating an autoimmune disorder in a host in need thereof.

17. The composition of claim 4 wherein said drug is cisplatin or a pharmaceutically acceptable salt thereof.

18. The composition of claim 17 wherein said cyclodextrin is beta-cyclodextrin; (O--R--Y)$^-$(Me)$^+$ is --O--$(CH_2)_4$--SO_3--Na^+, and n is about 7.

19. Use of a therapeutically effective amount of the composition of claim 17 in the manufacture of a medicament for treating cancer in a host in need thereof.

20. Use of a therapeutically effective amount of the composition of claim 17 in the manufacture of a medicament for inhibiting the growth of cancer in a host in need thereof.

21. The composition of claim 4 wherein said drug is doxorubicin or a pharmaceutically acceptable salt thereof.

22. The composition of claim 21 wherein said cyclodextrin is beta-cyclodextrin; (O--R--Y)$^{-}$(Me)$^{+}$ is --O--$(CH_2)_4$--$SO_3^{-}Na^{+}$, and n is about 7.

23. Use of a therapeutically effective amount of the composition of claim 21 in the manufacture of a medicament for treating cancer in a host in need thereof.

24. Use of a therapeutically effective amount of the composition of claim 21 in the manufacture of a medicament for inhibiting the growth of cancer in a host in need thereof.