US 20050014785A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0014785 A1**

Kastan et al. (43) **Pub. Date: Jan. 20, 2005**

(54) **TREATMENT OF RADIATION DISORDERS**

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(21) Appl. No.: **10/857,167**

(22) Filed: **May 27, 2004**

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/496,685, filed as 371 of international application No. PCT/US03/37838, filed on Nov. 26, 2003, which is a continuation of application No. 10/307,077, filed on Nov. 27, 2002.

Publication Classification

(51) **Int. Cl.**[7] **A61K 31/47**

(52) **U.S. Cl.** **514/313**

(57) **ABSTRACT**

The present invention provides methods and compositions for the treatment of DNA damage related disorders. One embodiment is a method for the inhibition of side effects associated with chemotherapeutic and radiotherapeutic agents using chloroquine compounds. Another embodiment is a method for treatment and/or prevention of lethal or sub-lethal radiation toxicities associated with terrorist acts or war.

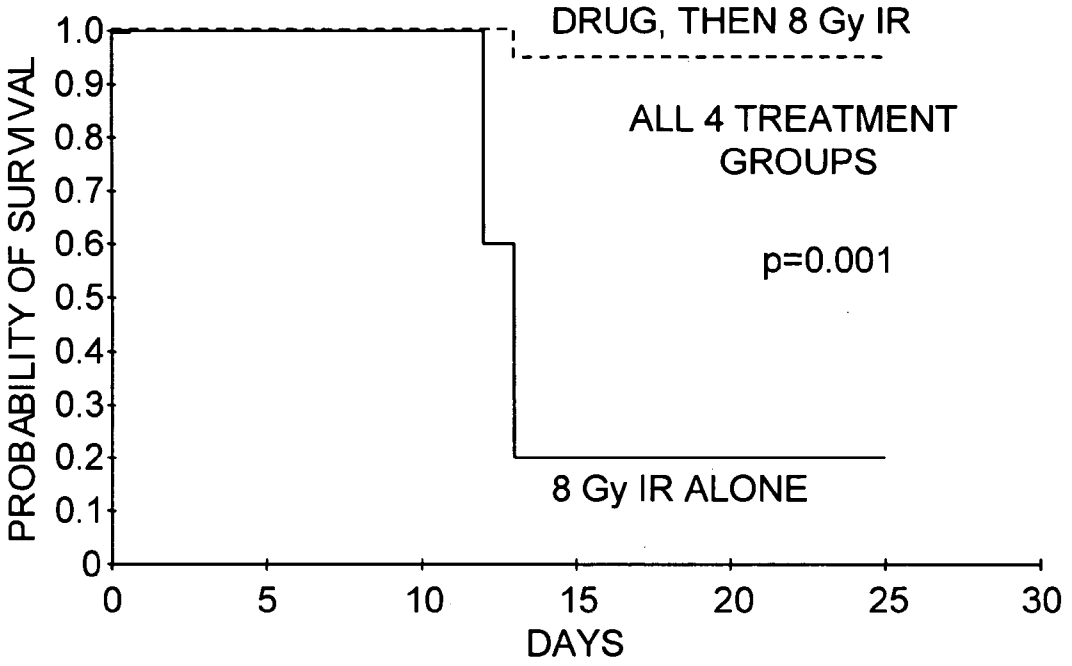

DRUG, THEN 8 Gy IR
ALL 4 TREATMENT GROUPS
p=0.001
8 Gy IR ALONE
PROBABILITY OF SURVIVAL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
DAYS

FIG. 1

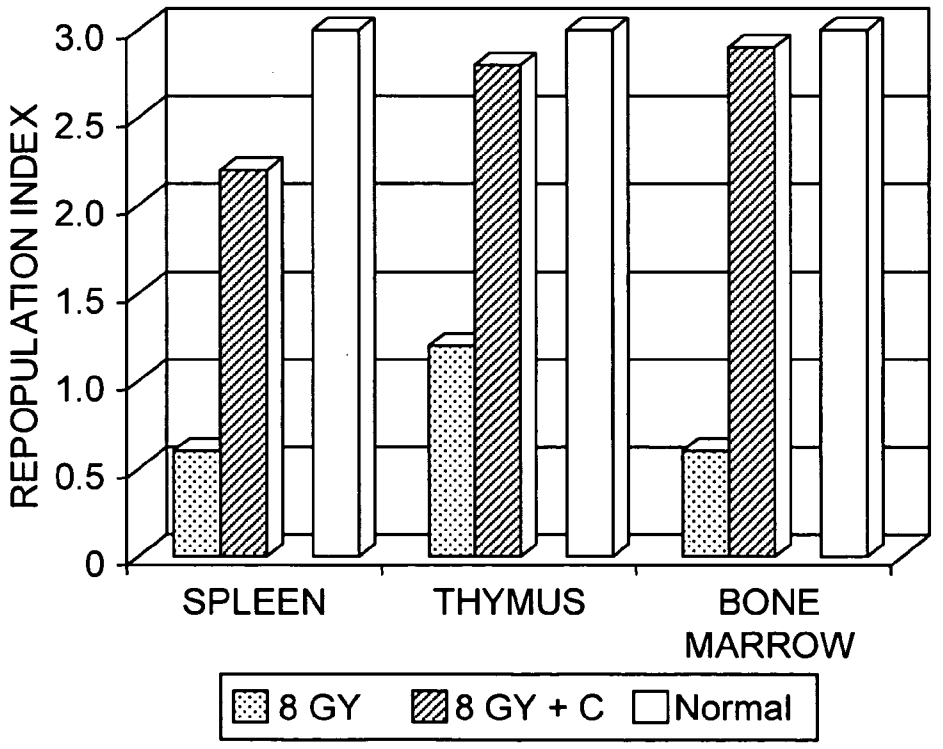

REPOPULATION INDEX
3.0
2.5
2.0
1.5
1.0
0.5
0
SPLEEN
THYMUS
BONE MARROW
8 GY
8 GY + C
Normal

FIG. 2

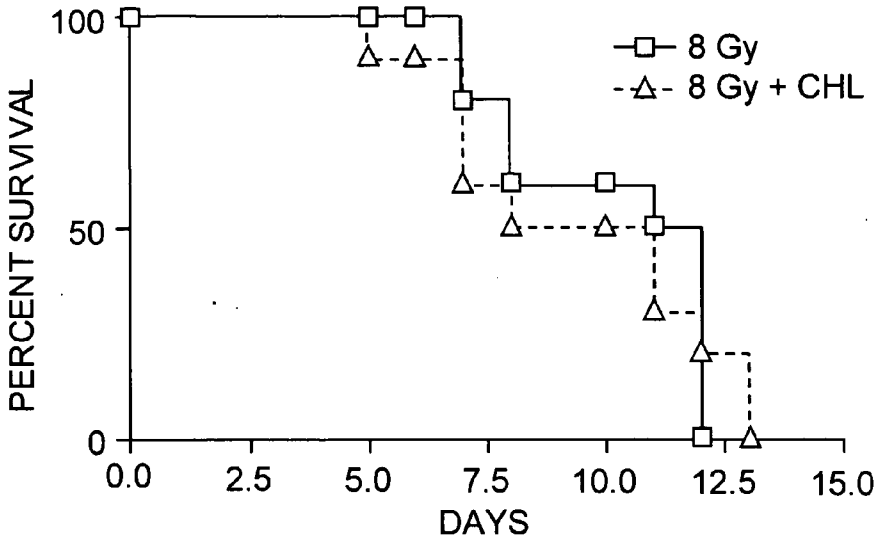

100
50
0
PERCENT SURVIVAL
8 Gy
8 Gy + CHL
0.0
2.5
5.0
7.5
10.0
12.5
15.0
DAYS

FIG. 3

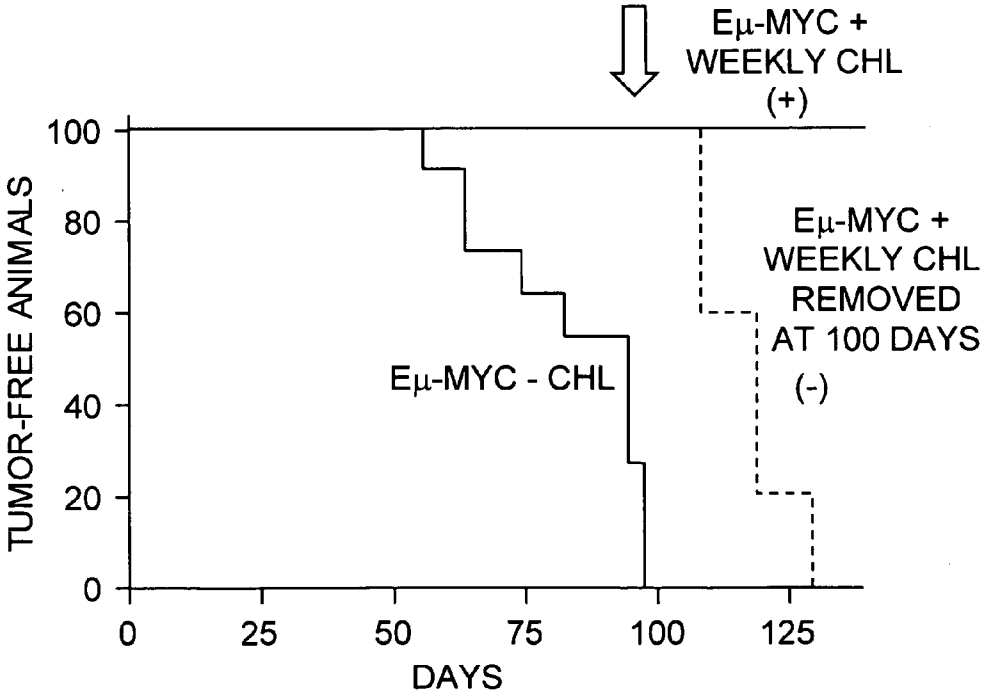

Eμ-MYC +
WEEKLY CHL
(+)
100
80
60
40
20
0
TUMOR-FREE ANIMALS
Eμ-MYC +
WEEKLY CHL
REMOVED
AT 100 DAYS
(-)
Eμ-MYC - CHL
0
25
50
75
100
125
DAYS

FIG. 4

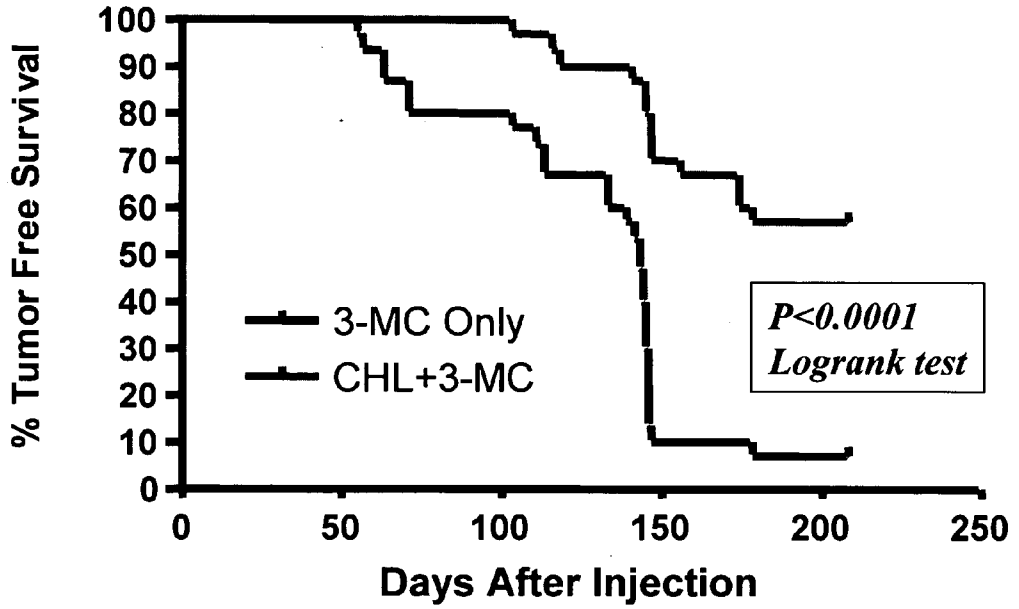

% Tumor Free Survival
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Days After Injection
3-MC Only
CHL+3-MC
P<0.0001
Logrank test

FIG. 5

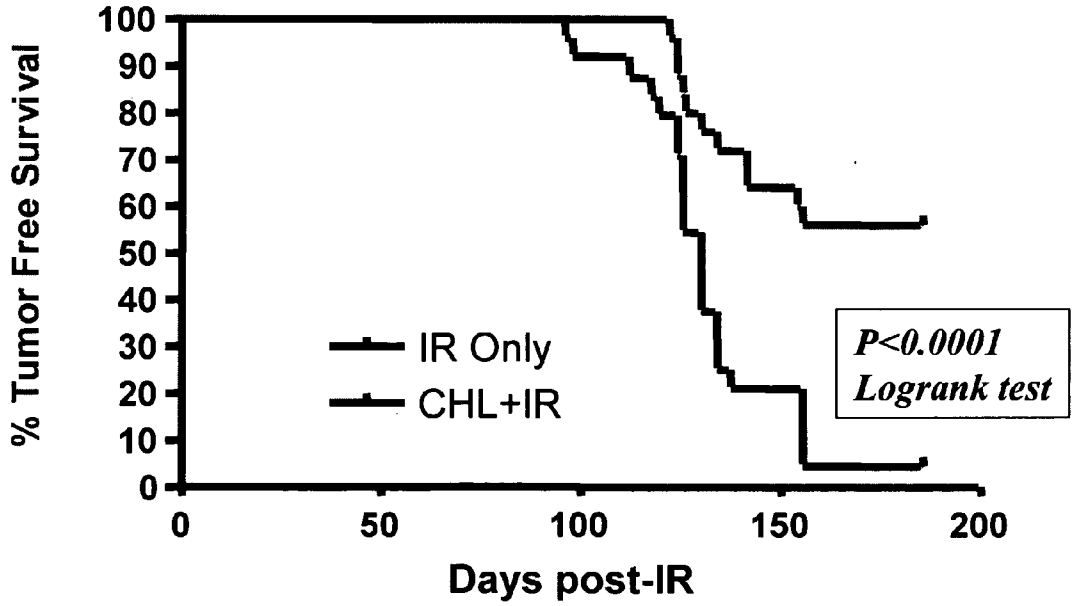

% Tumor Free Survival
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
Days post-IR
IR Only
CHL+IR
P<0.0001
Logrank test

FIG. 6

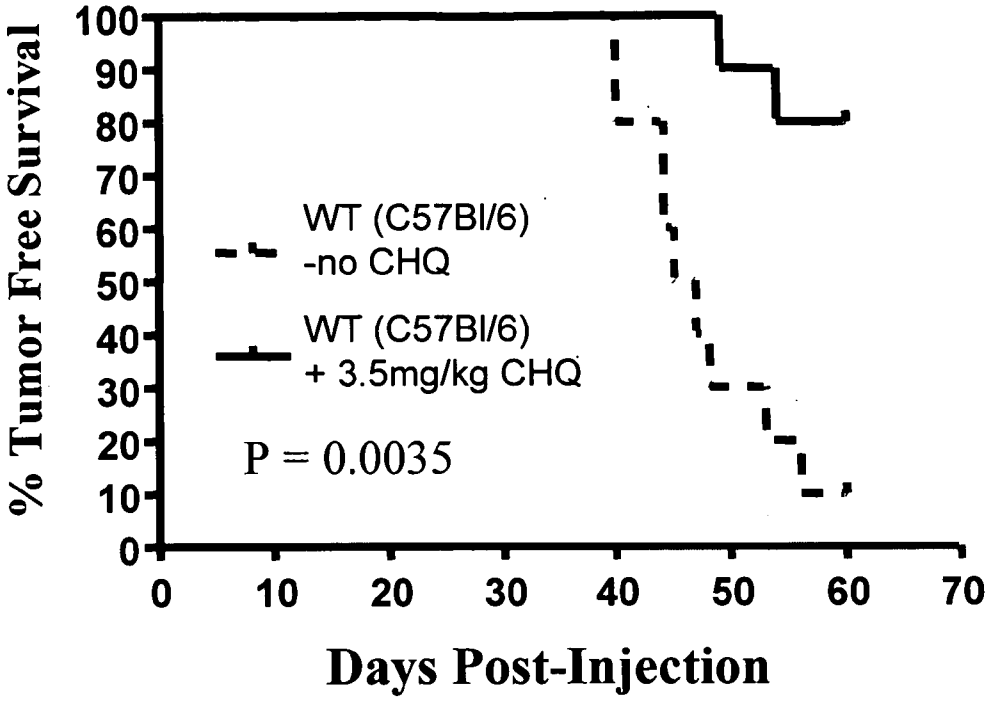

% Tumor Free Survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
WT (C57Bl/6)
-no CHQ
WT (C57Bl/6)
+ 3.5mg/kg CHQ
P = 0.0035
Days Post-Injection

FIG. 7

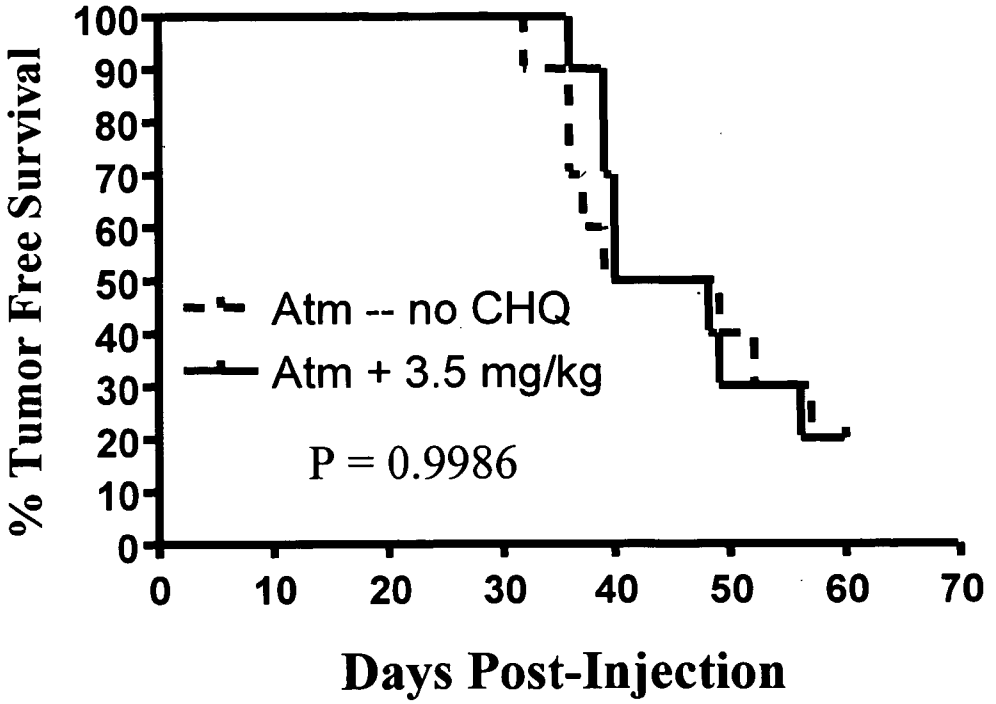

% Tumor Free Survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Atm -- no CHQ
Atm + 3.5 mg/kg
P = 0.9986
Days Post-Injection

FIG. 8

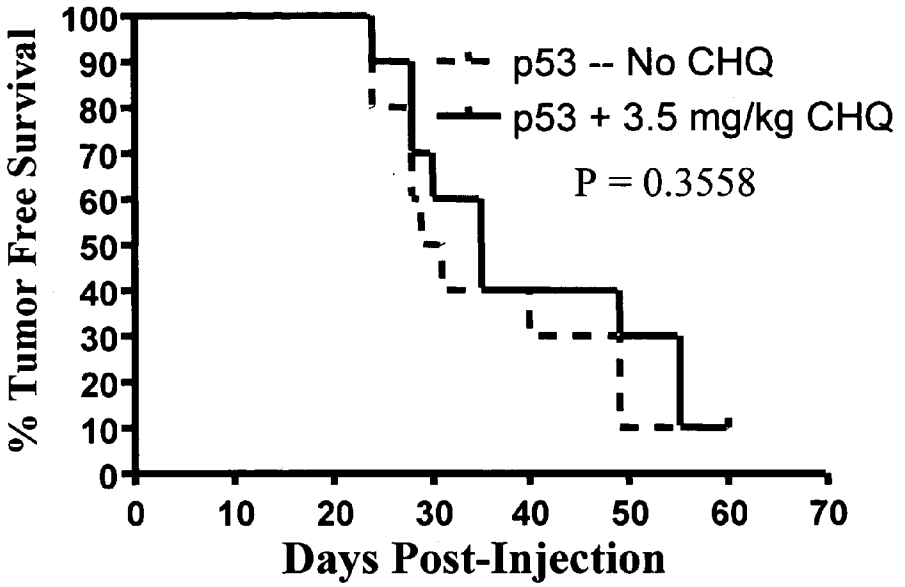

% Tumor Free Survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Days Post-Injection
p53 -- No CHQ
p53 + 3.5 mg/kg CHQ
P = 0.3558

FIG. 9

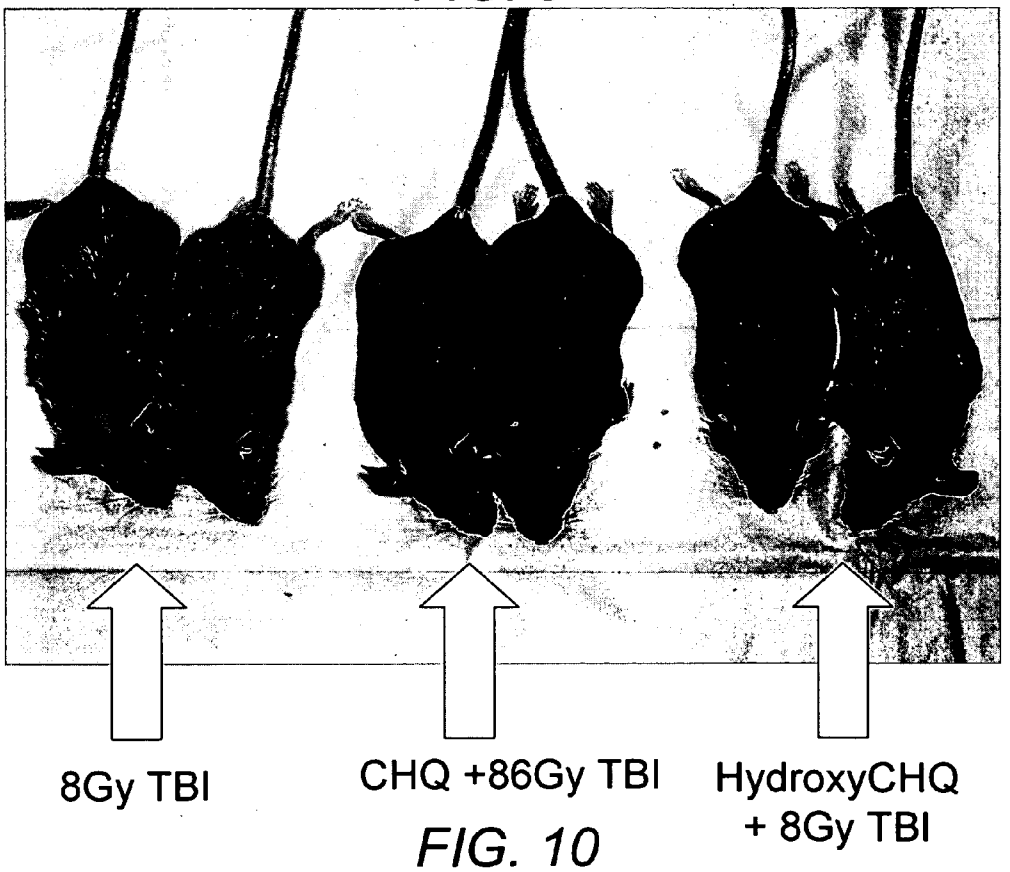

8Gy TBI
CHQ +86Gy TBI
HydroxyCHQ
+ 8Gy TBI

FIG. 10

TREATMENT OF RADIATION DISORDERS

RELATED APPLICATIONS

[0001] This application is a continuation-in-part application of U.S. patent application Ser. No. _____, filed on May 26, 2004, Attorney Docket No. 29202-710.831, which is a national stage application under 35 USC 371 of PCT/US03/37838 filed Nov. 26, 2003 which claims the benefit of priority from U.S. patent application Ser. No. 10/351,733 filed Jan. 24, 2003 and Ser. No. 10/307,077, filed Nov. 27, 2002 which are incorporated herein by reference in their entirety.

[0002] This invention was made in the course of research sponsored by the National Institutes of Health (NIH Grant Nos. CA71387). The U.S. government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Cancer is now the second leading cause of death in the United States. Over 1 million new cases of cancer are expected to be diagnosed in 2003 and over 500,000 people are expected to die of cancer.

[0004] Cancer is typically treated with one or a combination of three types of therapies: surgery, radiation, and chemotherapy. Overall costs for cancer, including treatments, were approximately $170 billion dollars in 2002. The cancer treatments are not only expensive; they are ineffective most of the time and also have many side effects. Hence, there is a demand for more effective cancer prevention and treatment agents, as well as for the prevention and treatment of DNA damage related conditions.

[0005] Radiation is all around us. Exposure to radiation includes radiation from natural sources and man-made sources. Acute and/or chronic exposure to radiation causes several adverse effects, including occasionally causing death. The current treatments for treating the adverse effects following exposure to radiation are ineffective most of the time and also have many side effects. Hence, there is a demand for more effective treatment of effects following exposure to radiation.

SUMMARY OF THE INVENTION

[0006] The present invention provides compositions, methods, and kits for the treatment and/or prevention of DNA damage related disorders, disorders caused by radiation exposure, and death associated with radiation exposure. In one embodiment, a chloroquine compound is administered for the prevention of DNA damage related disorders, like cancer and radiation related disorders. In another embodiment, the chloroquine compound does not prevent a localized skin cancer alone. In yet another embodiment, the cancers prevented by the chloroquine compounds are a localized skin cancer and a cancer that is not a localized skin cancer. Also described herein are methods of inhibiting the side effects of chemotherapeutic and/or radiotherapeutic agents using chloroquine compounds. Another embodiment is the prevention and/or treatment of lethal or sub-lethal radiation toxicities associated with acts of terrorism or war on response to such acts. The present invention also provides compositions, methods, and kits for use in the treatment of radiation related disorders.

BRIEF DESCRIPTION OF THE FIGURES

[0007] **FIG. 1** shows a Kaplan-Meier survival curve of C57/BL6 mice after exposure to 8 Gy total body irradiation (TBI). Half of the cohort received a dose of chloroquine (dashed line) by either i.p. injection (1.75 mg/kg or 3.5 mg/kg) or in their drinking water (3.5 mg/kg or 7 mg/kg) the day before the TBI. The one mouse which died in the chloroquine-treated group received 1.75 mg/kg by i.p. injection.

[0008] **FIG. 2** shows that chloroquine treatment enhances survival after TBI by enhancing recovery of hematopoietic progenitor cells. Five mice received 3.5 mg/kg chloroquine (C) by i.p. injection 24 and 4 hours prior to TBI (bars with diagonal stripes). Five mice received no chloroquine (stippled bars). Fourteen days after irradiation, the cellularity (open bars) of hematopoietic tissues (spleen, thymus, bone marrow) was assessed by a blinded observer on a scale of 0-3 with 3 being normal cellularity. The bars represent the average cellularity of the tissues from the 5 mice in each group.

[0009] **FIG. 3** shows a Kaplan-Meier survival curve of AT mice after exposure to 8 Gy TBI. Half of the cohort received a dose of 3.5 mg/kg chloroquine (CHL; dashed line) by i.p. injection 24 and 4 hours prior to the TBI.

[0010] **FIG. 4** demonstrates that chloroquine treatment prevents the development of tumors in Eμ-myc mice. After weaning, a cohort of transgenic mice expressing the c-myc oncogene were started on chloroquine (CHL) at 7.0 mg/kg in the drinking water ((+), solid line). Within 100 days, all of the mice with no drug in the water had died of leukemia, while none of the cohort of mice on drug had succumbed. The latter group of mice was then divided into two groups (timing of this event depicted by heavy arrow), one group of which was taken off of chloroquine ((−), dashed line) and the other group of which was started on i.p. injections of 3.5 mg/kg of chloroquine once a week. Within a month, all of the mice taken off of chloroquine had developed malignancies and all of the mice on the weekly i.p. injections remained tumor-free for months.

[0011] **FIG. 5** illustrates that chloroquine treatment reduces the development of tumors in mice injected with the potent chemical carcinogen, 3-methylcholanthrene (3-MC). Chloroquine (CHL, 3.5 mg/kg) was given by i.p. injection 24 and 4 hours prior to 3-MC injection in 30 mice and 30 mice received the carcinogen with no chloroquine pretreatment. The percentage of animals remaining tumor-free is plotted. Statistical significance, log rank test $P<0.0001$.

[0012] **FIG. 6** demonstrates that chloroquine treatment reduces the development of tumors in mice exposed to ionizing radiation in a protocol that induces thymic lymphomas. Chloroquine (CHL, 3.5 mg/kg) was given by i.p. injection 24 and 4 hours prior to irradiation in four successive weeks and animals were subsequently observed for the development of tumors. Statistical significance, log rank test $P=0.0012$.

[0013] **FIG. 7** shows tumor incidence in wildtype mice receiving either placebo or CHQ before 3-MC injection. CHQ markedly protects from tumor development.

[0014] **FIG. 8** shows tumor incidence in ATM-null mice receiving either placebo or CHQ before 3 MC injection. CHQ does not protect from tumor development.

[0015] **FIG. 9** shows tumor incidence in p53-null mice receiving either placebo or CHQ before 3 MC injection. CHQ does not protect from tumor development.

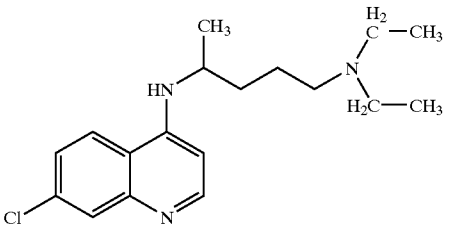
[0016] **FIG. 10** demonstrates the efficacy of two chloroquine compounds in preventing, in varying degree, the change in coat color in mice treated with 8 GY radiation.

DETAILED DESCRIPTION OF THE INVENTION

[0017] Chloroquine Compounds

[0018] The present invention provides methods, compositions, and kits for the prevention and/or treatment of DNA damage related disorders and death due to radiation exposure. Chloroquine compounds are useful in practicing the invention described herein. The term "chloroquine compounds" as used herein means chloroquine-like compounds, chloroquine and enantiomers, analogs, derivatives, metabolites, pharmaceutically acceptable salts, and mixtures thereof. Examples of chloroquine compounds include, but are not limited to, chloroquine phosphate, hydroxychloroquine, chloroquine diphosphate, chloroquine sulphate, hydroxychloroquine sulphate, and enantiomers, analogs, derivatives, metabolites, pharmaceutically acceptable salts, and mixtures thereof. The term "chloroquine-like compounds" as used herein means compounds that mimic chloroquine's biological and/or chemical properties.

[0019] In a specific embodiment, the invention is practiced with chloroquine. The chemical structure of chloroquine, N^4-(7-Chloro-4-quinolinyl)-N^1,N^1-diethyl-1,4-pentanediamine or 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline, is as follows:



[0020] Chloroquine (The Merck Index, p. 2220, 1996) is a synthetically manufactured drug containing a quinoline nucleus. Suitable synthesis techniques for chloroquine are well known in the art. For example see U.S. Pat. No. 2,233,970.

[0021] As mentioned above, the chloroquine compounds useful herein include chloroquine analogs and derivatives. A number of chloroquine analogs and derivatives are well known. For example, suitable compounds and methods for synthesizing the same are described in U.S. Pat. Nos. 6,417,177; 6,127,111; 5,639,737; 5,624,938; 5,736,557; 5,596,002; 5,948,791; 5,510,356; 2,653,940; 2,233,970; 5,668,149; 5,639,761; 4,431,807; and 4,421,920. In certain preferred embodiments, chloroquine is used in the methods described herein. In other embodiments hydroxychloroquine is used.

[0022] Examples of suitable chloroquine compounds include chloroquine phosphate; 7-chloro-4-(4-diethylamino-1-butylamino)quinoline (desmethylchloroquine); 7-hydroxy-4-(4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-butylamino-)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinol ine (hydroxychloroquine); 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; hydroxychloroquine phosphate; 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline (desmethylhydroxychloroquine); 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino-)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 8-[(4-aminopentyl)amino]-6-methoxydihydrochloride quinoline; 1-acetyl-1,2,3,4-tetrahydroquinoline; 8-[4-aminopentyl)amino]-6-methoxyquinoline dihydrochloride; 1-butyryl-1,2,3,4-tetrahydroquinoline; 7-chloro-2-(o-chlorostyryl)-4-[4-diethylamino-1-methylbutyl]aminoquiinolin e phosphate; 3-chloro-4-(4-hydroxy-.alpha., .alpha.'-bis(2-methyl-1-pyrrolidinyl)-2,5-xyl idinoquinoline, 4-[(4-diethylamino)-1-methylbutyl)amino]-6-methoxyquinoline; 3,4-dihydro-1 (2H)-quinolinecarboxyaldehyde; 1,1'-pentamethylenediquinoleinium diiodide; and 8-quinolinol sulfate, enantiomers thereof, as well as suitable pharmaceutical salts thereof.

[0023] Additional suitable chloroquine derivatives include aminoquinoline derivatives and their pharmaceutically acceptable salts such as those described in U.S. Pat. Nos. 5,948,791 and 5,596,002. Suitable examples include (S)—N_2-(7-Chloro-quinolin-4-yl)-N_1,N_1-dimethyl-propane-1,2-diamine; (R)—N_2-(7-chloro-quinolin-4-yl)-N_1,N_1-dimethyl-propane-1,2-diamine; N_1-(7-chloro-quinolin-4-yl)-2,N_2,N_2-trimethyl-propane-1,2-diamine; N_3-(7-chloro-quinolin-4-yl)-N_1,N_1-diethyl-propane-1,3-diamine; (RS)-(7-chloro-quinolin-4-yl)-(1-methyl-piperidin-3-yl)-amine; (RS)-(7-choro-quinolin-4-yl)-(1-methyl-pyrrolidin-3-yl)-amine; (RS)—N_2-(7-Chloro-quinolin-4-yl)-N_1,N_1-dimethyl-propane-1,2-diamine; (RS)—N_2-(7-chloro-quinolin-4-yl)-N_1,N_1-diethyl-propane-1,2-diamine; (S)—N_2-(7-chloro-quinolin-4-yl)-N_1,N_1-diethyl-propane-1,2-diamine; (R)—N_2-(7-chloro-quinolin-4-yl)-N_1,N_1-diethyl-propane-1,2-diamine; (RS)-7-chloro-quinolin-4-yl)-(1-methyl-2-pyrrolidin-1-yl-ethyl)-am ine; N_2-(7-chloro-quinolin-4-yl)-N_1,N_1-dimethyl-ethane-1,2-diamine; N_2-(7-chloro-quinolin-4-yl)-N_1,N_1-diethyl-ethane-1,2-diamine; N_3-(7-chloro-quinolin-4-yl)-N_1,N_1-dimethyl-propane-1,3-diamine; (R)—N_1-(7-chloro-quinolin-4-yl)-N_2,N_2-dimethyl-propane-1,2-diamine; (S)—N_1-(7-chloro-quinoline-4-yl)-N_2,N_2-dimethyl-propane-1,2-diamine; (RS)-(7-chloro-quinolin-4-yl)-(1-methyl-pyrrol idin-2-yl-methyl)-amine; N_1-(7-Chloro-quinolin-4-yl)-N_2-(3-chloro-benzyl)-2-methyl-propane-1,2-diamine; N_1-(7-chloro-quinolin-4-yl)-N_2-(benzyl)-2-methyl-propane-1,2-diamine; N_1-(7-chloro-quinolin-4-yl)-N_2-(2-hydroxy-3-methoxy-benzyl)-2-methyl-propane-1,2-diamine; N_1-(7-chloro-quinolin-4-yl)-N_2-(2-hydroxy-5-methoxy-benzyl)-2-methyl-propane-1,2-

diamine; and N_1-(7-chloro-quinolin-4-yl)-N_2-(4-hydroxy-3-methoxy-benzyl)-2-methyl-propane-1,2-diamine; (1S,2S)-N_1-(7-chloro-quinolin-4-yl)-N_2-(benzyl)-cyclohexane-1,2-diamine; (1S,2S)-N_1-(7-chloro-quinolin-4-yl)-N_2-(4-chlorobenzyl)-cyclohexane-1,2-diamine; (1S,2S)-N_1-(7-chloro-quinolin-4-yl)-N_2-(4-dimethylamino-benzyl)-cyclohexane-1,2-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(4-dimethylamino-benzyl)-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N-4-(benzyl)-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(3-chlorobenzyl)-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(2-hydroxy-4-methoxy-benzyl)-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(3,5-dimethoxy-benzyl)-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(4-methylsulphanyl-benzyl)-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(4-diethylamino-benzyl)-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(biphenyl-4-yl)methyl-cyclohexane-1,4-diamine; trans-N_1-(7-chloro-quinolin-4-yl)-N_4-[2-(3,5-dimethoxy-phenyl)-ethyl]-cyclohexane-1,4-diamine; cis-N_1-(7-chloro-quinolin-4-yl)-N_4-(4-methoxy-benzyl)-cyclohexane-1,4-diamine; trans-N_1-(7-chloro-quinolin-4-yl)-N_4-(4-dimethylamino-benzyl)-cyclohexane-1,4-diamine; and trans-N_1-(7-chloro-quinolin-4-yl)-N_4-(2,6-difluoro-benzyl)-cyclohexane-1,4-diamine.

[0024] Chloroquine compounds such as chloroquine may exhibit the phenomena of tautomerism, conformational isomerism, geometric isomerism, and/or optical isomerism. The invention covers any tautomeric, conformational isomeric, optical isomeric and/or geometric isomeric forms of the chloroquine compounds, as well as mixtures of these various different forms.

[0025] Chloroquine and hydroxychloroquine are generally racemic mixtures of (−)- and (+)-enantiomers. The (−)-enantiomers are also known as (R)-enantiomers (physical rotation) and 1-enantiomers (optical rotation). The (+)-enantiomers are also known as (S)-enantiomers (physical rotation) and r-enantiomers (optical rotation). The metabolism of the (+)- and the (−)-enantiomers of chloroquine are described in Augustijins and Verbeke (1993) *Clin. Pharmacokin.* 24(3):259-69; Augustijins, et al. (1999) *Eur. J. Drug Metabol. Pharmacokin.* 24(1):105-8; DuCharme and Farinotti (1996) *Clin. Pharmacokin.* 31(4):257-74; Ducharme, et al. (1995) *Br. J. Clin. Pharmacol.* 40(2):127-33. Preferably, the (−)-enantiomer of chloroquine is used. In other embodiments, the (+) enantiomer of chloroquine is employed.

[0026] In certain embodiments, essentially pure (+) or (−) enantiomers are used in the methods described herein. The term "essentially pure" does not require a 100% enantiomer. The term refers to about more than 95% pure enatiomer, preferably more than about 90%, more preferably more than about 85% pure, even more preferably more than about 80%, and most preferably more than 75% pure. In certain embodiments mixtures containing various ratios of the (+) or (−) form in combination with the racemic mixture are used. In certain embodiments employing the (+) or (−) forms, the doses of the enantiomer used can be less than if the racemic mixture were used. The enantiomers of chloroquine and hydroxychloroquine may be prepared by procedures known to the art.

[0027] The chloroquine compounds may metabolize to produce active metabolites. The used of active metabolites is also within the scope of the present invention.

[0028] Not intending to be limited by one mechanism, it is believed that the chloroquine compounds and chloroquine-like compounds act by enhancing the activity of Ataxia-Telangiectasia Mutated (ATM) kinase and downstream events. The agonistic properties of chloroquine on ATM kinase have been demonstrated. Hence, it is intended herein that choroquine-like compounds include compounds that are agonists of ATM kinase. Agonists of ATM kinase include compounds that promote the dissociation of ATM into active monomers and/or compounds that promote phosphorylation of a serine corresponding to the residue 1981 of ATM kinase of SEQ ID NO:1.

[0029] Use of Chloroquine Compounds

[0030] In one aspect, the invention provides methods of treating an animal subject, including a human. The term "animal subject" as used herein includes humans as well as other mammals. The methods described herein generally involve the administration of effective amounts of chloroquine compounds and/or chloroquine like compounds for the treatment and/ore prevention of DNA damage related disorders. The term "DNA damage related disorders" include, but are not limited to, cancer, aging, disorders caused by damage to DNA due to exposure to carcinogens, toxins, free radicals, like oxygen radical, or DNA damaging radiations like ionizing radiation and UV radiation. The chloroquine compounds are also useful for prevention of tissue injury resulting from ischemia, such as that which occurs following myocardial infarction or stroke. The effects of the chloroquine compounds used in the methods described herein include systemic, local, and topical effects. It is preferred that the effects of the chloroquine compounds in the methods described herein are systemic.

[0031] In one embodiment, the chloroquine compounds are used as prophylactics to prevent DNA damage related disorders. The chloroquine compounds are useful in the prevention of cancers caused by toxins, carcinogens, DNA damaging radiations, and/or genetic mutations. For example, chloroquine compounds are useful in the prevention of cancers caused by exposure to toxins and carcinogens like aromatic hydrocarbons, cigarette smoke, acetyl amino fluorine, MTBE, etc. Also, chloroquine compounds are useful in prevention of cancers caused by DNA damaging radiations like UV and ionizing radiation. The ionizing radiations includes both natural and therapeutic radiation exposures. Examples of ionizing radiations are X-rays for diagnostics and radiation therapy used for tumors and unintended exposure to radiation as an act of terrorism or war.

[0032] The prophylactic uses for cancer described herein are not envisioned to encompass the prevention solely of localized skin carcinomas like basal cell epithelioma and squamous cell carcinoma, skin carcinomas, Burkitt's lymphoma, or skin pathologies caused by harmful radiation. When used in patients with actinic keratosis, it is envisioned the chloroquine compounds do not solely inhibit basal cell epithelioma and squamous cell carcinoma. In one embodiment, the chloroquine compounds are used to prevent a localized skin carcinoma and at least one cancer that is not a localized skin carcinoma. Examples of cancers that are not localized skin carcinomas include, but are not limited to,

melanomas, lymphomas, prostate cancer, breast cancer, colon cancer, lung cancer, retinoblastoma, neuroblastoma, sarcomas, and ovarian cancer.

[0033] In a preferred embodiment, chloroquine compounds are used in the prevention of one or more of the following cancers—melanomas, prostate cancer, breast cancer, colon cancer, lung cancer, non-Hodgkins lymphoma, retinoblastoma, neuroblastoma, sarcomas, and ovarian cancer.

[0034] The chloroquine compounds can be used to prevent secondary cancers, i.e., cancers that are caused by radiation therapy and chemotherapy used to treat the primary cancer. In one embodiment, the chloroquine compounds are used to prevent the occurrence of breast cancer in patients receiving radiation therapy for non-Hodgkin's lymphoma. Also, in these patients the chloroquine compounds can be used to inhibit the cellular damage caused by the radiation therapy to normal cells and enhance the repair process of the normal cells. The chloroquine compounds are also suitable for prevention of the reoccurrence of cancers in patients who have had prior incidences of cancer.

[0035] In one embodiment, the chloroquine compounds are administered to decrease or prevent the side-effects of radiation therapy used to treat cancer. The chloroquine compounds can be administered prior to, during, or after treatment with radiation. In this embodiment, the beneficial effect of the chloroquine compounds is contemplated to be not solely limited to a beneficial effect on pathological skin conditions like skin carcinomas and dermatoses. The use of chloroquine compounds in combination with radiation therapy is contemplated to protect the normal cells and inhibit the cellular damage caused by the radiation therapy to normal cells and enhance the repair process of the normal cells.

[0036] In one embodiment, the chloroquine compounds are used in immunosuppressed patients, like transplant patients. In immunosuppressed patients, the chloroquine compounds can be used to prevent cancers. The chloroquine compounds can be used to prevent Epstein Barr virus induced lymphoproliferative syndrome.

[0037] In another embodiment, chloroquine compounds are used as prophylactics to inhibit side effects of frequent exposure to X-rays in athletes. This method would also be useful for other patient populations that are frequently exposed to DNA damaging radiations, such as X-ray technicians, pilots, police officers, astronauts, and the like. It is known that exposure to X-rays causes DNA damage. Administration of chloroquine compounds is contemplated to inhibit the side-effects of frequent exposure to DNA damaging radiations, including inhibiting the damage to cells due to damage to DNA.

[0038] The present invention also provides methods for preventing DNA damage, inhibiting the effects of DNA damage, and stimulating cellular response to DNA damage by administering an effective amount of chloroquine compounds. Not intending to be limited by one mechanism of action, it is contemplated that cellular responses are enhanced by an agonistic activity on ATM kinase by priming the cell to respond to agents which cause DNA damage. Further details on ATM kinase are provided in International PCT application no. US03/38091 filed Nov. 26, 2003, which is incorporated by reference herein in its entirety.

[0039] The prophylactic benefits of chloroquine compounds can be obtained by administering in advance of exposure to the DNA damaging agent to provide the enhancing effect in one embodiment. The amount of time prior to the exposure to the DNA damaging agent that the chloroquine compound is administered can vary from days, hours, to minutes. Also, the chloroquine compounds can be administered during exposure to the DNA damaging agent or after such exposure. In one embodiment, the effective amount of a chloroquine compound is an amount which reduces DNA damage, reduces DNA mutation or increases survival of cells exposed to a DNA damaging agent when compared to cells exposed to the same DNA damaging agent and not receiving a chloroquine compound. In another embodiment, the effective amount of a chloroquine compound is an amount which produces anti-oxidant effects.

[0040] The prophylactic use of chloroquine includes the prevention of tissue injury resulting from ischemia, such as that which occurs following myocardial infarction or stroke. While not intending to be limited to one mechanism of action, it is believed that the chloroquine compounds prevent cellular death due to oxidative damage during reperfusion and as such can ameliorate tissue injury resulting from ischemic injury.

[0041] In one embodiment, chloroquine compounds are used in the treatment of DNA damage related disorders. The chloroquine compounds are used preferably in combination with chemotherapeutic or radiotherapeutic agents to prevent the side-effects associated with the chemotherapeutic agents. It is known that chloroquine compounds can inhibit multiple drug resistance. Hence, it is not intended that the methods described herein produce a beneficial effect on multiple drug resistance alone. In a preferred embodiment, the beneficial effects of chloroquine compounds, when used in combination with chemotherapeutic agents, are due to modulation of ATM kinase activity. It is contemplated that the chloroquine compounds protect the normal cells and inhibit the cellular damage caused by the radiation therapy to normal cells and enhance the repair process of the normal cells.

[0042] In one embodiment, the chloroquine compounds are used to treat and/or prevent disorders caused by oxidative damage. The chloroquine compounds can be administered with anti-oxidants, like vitamin B12, to stimulate the cellular response to DNA damage and promote the repair of the cells exposed to the oxidative agents.

[0043] Treatment of Radiation Therapy Related Adverse Effects

[0044] In one aspect of the invention the chloroquine compounds are used in the treatment of adverse effects associated with radiation therapy. The chloroquine compounds may be used to therapeutically treat the adverse effects of radiation therapy and/or prophylactically to prevent the occurrence of the adverse effects associated with radiation therapy. In preferred embodiments, the use of the chloroquine compounds does not adversely affect the efficacy and/or potency of the radiation therapy in the disease being treated.

[0045] Radiation therapy, also known as radiotherapy, x-ray therapy, or irradiation, is the treatment of disease using penetrating beams of high-energy or low-energy waves or streams of particles called radiation. The radiation is used

for the treatment of cancer and is usually administered from special machines or from radioactive substances. The doses of radiation that damage or destroy the diseased cells, such as cancer cells, can also injure or kill normal cells. These effects of radiation on normal cells cause treatment side effects. In one embodiment, the chloroquine compounds described herein are used in the treatment of adverse effects associated with radiation therapy. The chloroquine compounds can be administered prior to the radiation therapy or after the radiation therapy is started. Preferably the chloroquine compounds minimize the effects of the radiation therapy on normal cells.

[0046] The high energy rays used for radiation therapy can include for example, x-rays, an electron beam, or cobalt-60 gamma rays. Also beams of protons or neutrons may be used for radiation therapy. Internal radiation therapy places the radiation source as close as possible to the diseased cells. Some of the radioactive substances used for internal radiation treatment include cesium, iridium, iodine, phosphorus, and palladium.

[0047] Side effects of treatment with radiation include temporary or permanent loss of hair in the area being treated, skin irritation, temporary change in skin color in the treated area, and tiredness. Some people who receive radiation to the head and neck experience redness and irritation in the mouth, a dry mouth, difficulty in swallowing, changes in taste, or nausea. Other possible side effects include a loss of taste, earaches, and swelling. Radiation therapy can cause hair loss (alopecia). Radiation therapy can also cause low white blood cell counts or low levels of platelets. Neutropenia, which refers to an abnormally low number of neutrophils in the blood, can also be an adverse effect associated with radiation therapy.

[0048] In certain embodiments, the chloroquine compounds are used to treat the adverse effects of radiation therapy on the skin. In other embodiments, the compounds are used to treat alopecia associated with radiation therapy. In other preferred embodiments, the chloroquine compounds are used to treat the side effects associated with blood, such as neutropenia.

[0049] Treatment of Radiation Related Disorders

[0050] In one aspect of the invention the chloroquine compounds are used in the treatment of radiation-related disorders.

[0051] Radiation is typically classified as non-ionizing and ionizing radiation. Examples of sources of non-ionizing radiation include, but are not limited to, power lines, AM/FM radio and televsion, microwave oven, heat lamps, and tanning salons. In one embodiment, chloroquine compounds are used to treat adverse health effects caused by non-ionizing radiations. The compounds can be used therapeutically and/or prophylactically.

[0052] The kinds of ionizing radiation include alpha particles, beta particles, gamma rays, and x-rays. Ionizing radiations have enough energy to break chemical bonds and typically cause biological damage by breaking and/or damaging DNA bonds. In preferred embodiments, the chloroquine compounds are used to treat adverse health effects caused by ionizing radiations. The compounds can be used therapeutically and/or prophylactically.

[0053] Typically, due to the biological effects of radiation, cells either die or due to the damage to the DNA the cells may mutate. The mutations can be such that the effects of the mutation are seen immediately or after several days, months, or years. Also, the mutations could be passed on the affected individuals offsprings or may show up many generations later. In certain embodiments, the use of chloroquine has a beneficial effect on the cell death and/or the mutations of the cells following exposure to radiation. For example, use of chloroquine prior to exposure to radiation and/or following to radiation can decrease the number of mutations and thus cause a decrease in the adverse genetic effects caused by exposure to radiation.

[0054] Acute radiation syndrome (ARS) is caused by exposure of the body to high doses of radiation usually over a short period of time. The symptoms include nausea, vomiting, diarrhea, loss of appetite, fatigue, fever, diminished organ function, and possibly even seizures, coma, and death. ARS also typically includes skin damage, such as swelling, itching, redness of skin, and hair loss. In some embodiments, chloroquine compounds are used therapeutically and/or prophylactically for ARS. In the treatment of ARS, in some embodiments, the beneficial effects are not solely on the skin damage caused by radiation. Preferably, the chloroquine compounds have a beneficial effects on both the skin damage and one or more of the non-skin related symptoms of ARS, such as the nausea, vomiting, diarrhea, loss of appetite, fatigue, fever, diminished organ function, seizures and coma.

[0055] Exposure to radiation can also cause cancers, such as leukemia, breast, bladder, colon, liver, lung, esophagus, ovarian, multiple myeloma, and stomach cancers. Other cancers that can be caused by radiation include, prostate, nasal cavity/sinuses, pharyngeal, and laryngeal, and pancreatic cancer. In some embodiments, chloroquine compounds are used therapeutically and/or prophylactically for cancers caused by exposure to radiation.

[0056] In some embodiments, the chloroquine compounds are used by astronauts, prior to or during space flight, to reduce the adverse effects caused by exposure to radiation during space flights. In other embodiments, the methods described herein are employed for the treatment of hospital personnel who are exposed to X-ray radiation. In yet other embodiments, personnel who are exposed to radiation in war situations or have to work in areas with abnormally high levels of natural radiation are treated with the chloroquine compounds. Also, the compounds and methods described herein can be used to treat personnel who are involved in clean-up operations following an accidental or intentional, e.g., a terrorist attack, release of radiation.

[0057] In some embodiments, the chloroquine compounds can be used for treatment in anticipation of or following exposure to a "dirty bomb." A "dirty bomb" typically refers to a bomb that combines conventional explosives, such as dynamite, with radioactive materials in the form of powder or pellets. Also, the chloroquine compounds can be used in the therapeutic and/or prophylactic treatment of medical and other personnel who would be involved in the treatment and clean-up operations following the explosion of a dirty bomb. In certain embodiments, hospitals, pharmacies, and non-medical personnel stockpile chloroquine compounds to be used in the event of an explosion of a dirty bomb. The

chloroquine compounds from such a stockpile can be used by subjects exposed to the radiation from the dirty bomb and subjects likely to exposed to the radiation.

[0058] In certain other embodiments, the chloroquine compounds are used for the prevention of death and/or for the prophylactic treatment of soldiers and other personnel entering areas with expected weapons of mass destruction or expected to encounter radiation exposure due to an act of war or terrorism.

[0059] In certain preferred embodiments, chloroquine and hydroxychloroquine are used in the treatment of radiation related disorders. Even more preferred is the use of chloroquine. In other embodiments, the pure enantiomers of chloroquine or hydroxychloroquine, either the (+) or (−) form are used.

[0060] Therapeutic and Prophylactic Benefits

[0061] In one embodiment, the chloroquine compounds are used as prophylactic agents. For prophylactic benefit, the chloroquine compound may be administered to a patient at risk of developing a DNA damage related disorder like cancer or to a patient reporting one or more of the physiological symptoms of a DNA damage related disorder, even though a diagnosis of such disorder may not have been made. A prophylactic benefit is achieved when a disorder is prevented from afflicting a patient. This prevention can include the affliction of the patient with a milder form of the disorder or the appearance of fewer or no symptoms of the disorder being prevented or the absence of the disorder in the patient being treated.

[0062] In addition to a prophylactic benefit, the chloroquine compounds can be used for their therapeutic benefits. In one embodiment, the chloroquine compounds are used to treat DNA damage related disorders. In a preferred embodiment, the beneficial effect of the chloroquine compounds is not due to an inhibition of multiple drug resistance. The term "treating" as used herein includes achieving a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated. For example, in a cancer patient, therapeutic benefit includes eradication or amelioration of the underlying cancer. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the patient, notwithstanding that the patient may still be afflicted with the underlying disorder. For example, administration of a chloroquine compound to a patient suffering from cancer provides therapeutic benefit not only when the patient's tumor marker level is decreased, but also when an improvement is observed in the patient with respect to other complications that accompany the cancer like pain and psychiatric disorders.

[0063] Effective Amount

[0064] A physician or veterinarian having ordinary skill in the art may readily determine and prescribe the effective amount of the chloroquine compound required in the methods described herein. Pharmaceutical compositions suitable for use in the present invention include compositions wherein the chloroquine compound and other optional active ingredients are present in an effective amount. The effective amounts include doses that partially or completely achieve the desired therapeutic, prophylactic, and/or biological effect. The actual amount effective for a particular application will depend on the condition being treated and the route of administration. Determination of an effective amount is well within the capabilities of those skilled in the art, especially in light of the disclosure herein.

[0065] The effective amount for use in humans can be determined from animal models. For example, a dose for humans can be formulated to achieve circulating and/or gastrointestinal concentrations that have been found to be effective in animals.

[0066] In one embodiment, the effective amount can include the dose ranges, modes of administration, formulations, etc., that have been recommended or approved by any of the various regulatory or advisory organizations in the medical or pharmaceutical arts (eg, FDA, AMA) or by the manufacturer or supplier. Effective amounts of chloroquine can be found, for example, in the Physicians Desk Reference.

[0067] The daily dosage range of chloroquine, in one embodiment, can vary between about 0.1 mg/kg to about 2 gm/kg body weight. The daily dose of a chloroquine compound may be less than about 2 gm/kg, less than about 1.5 gm/kg, or less than about 1 gm/kg. In one embodiment, the daily dose of a chloroquine coumpound is more than about 0.5 mg/kg, more than about 500 mg/kg, or more than about 1 gm/kg. Preferred daily dosage ranges of a chloroquine compound are about 0.5 mg/kg to about 50 mg/kg or about 1.0 mg/kg to about 10 mg/kg or about 30 mg/kg to about 50 mg/kg body weight. Preferred doses of chloroquine diphosphate and hydroxychloroquine are about 3.5 mg/kg and 7.0 mg/kg.

[0068] The dosage can vary depending on the subject being treated. For example, a preferred dosage in mice is 3.5 mg/kg once or twice a day. The equivalent dosages in monkeys and humans are shown in the table below.

Mouse (20 g)	Monkey (3.0 kg)	Man (60 kg)	Man (60 kg) CHG Equivalent
3.5 mg/kg	0.875 mg/kg	0.292 mg/kg	17.5 mg CHQ
7.0 mg/kg	1.75 mg/kg	0.583 mg/kg	35.0 mg CHQ

[0069] Preferred dosages ranges in human are from 0.05-1 mg/kg, more preferably 0.1 to 0.8 mg/kg, more preferably 0.2-0.6 mg/kg. In patients whose risk to cancer is occasioned by a distinct event (e.g., exposure to carcinogen or radiation), the dosage is preferably administered daily before, during and/or immediately following the event, for a total period of at least 1 day, 3 days, a week or a month. For example, if the risk of exposure is known in advance, an exemplary regime entails administering the chloroquine compound on the day before, the day of exposure and the day after exposure. If the risk of exposure is not known in advance, an exemplary regime entails administering the chloroquine compound at least one the day of exposure and the day following exposure. For patients subject to a chronic risk (e.g., through genetic variation), the dosage is preferably administered weekly for an indefinite period. The dosage range can be lower e.g., 0.05-0.2 mg/kg per day or

per week of chloroquine if a purified enantiomer is used, such as the purified (−) form or the purified (+) form.

[0070] In some embodiments, the effective amount of chloroquine is administered once a month, every other week, once a week, more than once a week, or once a day. The dose of chloroquine can be administered once or more than once a day. In yet another embodiment, the effective amount of a chloroquine compound is an amount that produces the intended beneficial effects but does not produce the side-effects associated with chloroquine compounds, like retinoblastoma.

[0071] In one embodiment, the invention provides a kit comprising a chloroquine compound packaged in association with instructions teaching a method of using the compound according to one or more of the above-described methods. The kit can contain the chloroquine compound packaged in unit dosage form.

[0072] Routes of Administration and Formulation

[0073] The compounds useful in the present invention, or pharmaceutically acceptable salts thereof, can be delivered to the patient using a wide variety of routes or modes of administration. Suitable routes of administration include, but are not limited to, inhalation, transdermal, oral, rectal, transmucosal, intestinal and parenteral administration, including intramuscular, subcutaneous and intravenous injections.

[0074] The formulations useful herein can administer the chloroquine compounds topically or systemically. In one embodiment, the formulation of chloroquine compound is administered systemically. In another embodiment, the formulation of chloroquine compound has a systemic effect if administered either topically or systemically.

[0075] The term "pharmaceutically acceptable salt" means those salts which retain the biological effectiveness and properties of the compounds used in the present invention, and which are not biologically or otherwise undesirable. Such salts include salts with inorganic or organic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, nitric acid, sulfuric acid, methanesulfonic acid, p-toluenesulfonic acid, acetic acid, fumaric acid, succinic acid, lactic acid, mandelic acid, malic acid, citric acid, tartaric acid or maleic acid. In addition, if the compounds used in the present invention contain a carboxy group or other acidic group, it may be converted into a pharmaceutically acceptable addition salt with inorganic or organic bases. Examples of suitable bases include sodium hydroxide, potassium hydroxide, ammonia, cyclohexylamine, dicyclohexylamine, ethanolamine, diethanolamine and triethanolamine.

[0076] If necessary, the compounds and useful herein may be administered in combination with other therapeutic agents. The choice of therapeutic agents that can be co-administered with the compounds of the invention will depend, in part, on the condition being treated.

[0077] Agents used in accordance with the methods of the invention may be conveniently administered in a pharmaceutical composition containing the active compound in combination with a suitable carrier. Such pharmaceutical compositions may be prepared by methods and contain carriers which are well-known in the art. A generally recognized compendium of such methods and ingredients is Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro, editor, 20th ed. Lippingcott Williams & Wilkins: Philadelphia, Pa., 2000. A pharmaceutically-acceptable carrier, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, is involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be acceptable in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

[0078] Examples of materials which may serve as pharmaceutically-acceptable carriers include sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; lycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in harmaceutical formulations. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0079] Agents of use in the invention may be administered parenterally (for example, by intravenous, intraperitoneal, subcutaneous or intramuscular injection), topically (including buccal and sublingual), orally, intranasally, intravaginally, or rectally, with oral administration being particularly preferred.

[0080] For oral therapeutic administration, the composition may be combined with one or more carriers and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums, foods and the like. Also, for oral consumption the active ingredient may be dissolved or suspended in water or other edible oral solutions. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 0.1 to about 100% of the weight of a given unit dosage form. The amount of active agent in such therapeutically useful compositions is such that an effective dosage level will be obtained.

[0081] The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. The above listing is merely representative and one skilled in the art could envision other binders, excipients, sweetening

agents and the like. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like.

[0082] For administration orally, the compounds may be formulated as a sustained release preparation. Numerous techniques for formulating sustained release preparations are described in the following references—U.S. Pat. Nos. 4,891,223; 6,004,582; 5,397,574; 5,419,917; 5,458,005; 5,458,887; 5,458,888; 5,472,708; 6,106,862; 6,103,263; 6,099,862; 6,099,859; 6,096,340; 6,077,541; 5,916,595; 5,837,379; 5,834,023; 5,885,616; 5,456,921; 5,603,956; 5,512,297; 5,399,362; 5,399,359; 5,399,358; 5,725,883; 5,773,025; 6,110,498; 5,952,004; 5,912,013; 5,897,876; 5,824,638; 5,464,633; 5,422,123; and 4,839,177; and WO 98/47491. These references are hereby incorporated herein by reference in their entireties. In a preferred embodiment, the sustained release formulation utilized has an enteric coating.

[0083] For administration by inhalation, the active compound(s) may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., 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. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0084] A syrup or elixir may contain the active agent, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active components may be incorporated into sustained-release preparations and devices including, but not limited to, those relying on osmotic pressures to obtain a desired release profile. Once daily formulations for each of the active components are specifically included.

[0085] The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0086] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or transcutaneous delivery (for example subcutaneously or intramuscularly), intramuscular injection or a transdermal patch. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0087] The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well-known in the medical arts.

[0088] The invention is described in greater detail by the following non-limiting examples.

EXAMPLES

Example 1

Radioprotection Assay

[0089] HeLa cells were treated with 2 μg/ml of chloroquine for one hour, washed for one hour, and irradiated at 2 or 6 Gy. Subsequently, 1000 cells were plated and assessed for colony formation. Table 1 shows that exposure to chloroquine prior to irradiation increased cell survival by 30%.

TABLE 1

Treatment	Average Number of Colonies*	Standard Deviation
2 Gy	444	19.5
Chloroquine + 2 Gy	580	21.2
6 Gy	94.6	10.6
Chloroquine + 6 Gy	129	8.6

*Averages were from five individual samples.

[0090] To test the possibility that chloroquine activation of ATM may cause radioprotection, C57/BL6 mice were exposed to 8 Gy IR, a dose which kills approximately 80% of the mice at around two weeks. Death appears to result from hematopoietic toxicities. The day before total body irradiation (TBI), mice were either given an i.p. injection of chloroquine or chloroquine was added to the drinking water (5 mice—i.p. 1.75 mg/kg chloroquine; 5 mice—i.p. 3.5 mg/kg chloroquine; 5 mice —1.75 mg/kg chloroquine in drinking water; 5 mice —3.5 mg/kg chloroquine in drinking water). **FIG. 1** shows a Kaplan-Meier survival curve indicating that a dose of chloroquine prior to the TBI provided significant protection from death. Significant protection was not observed in ATM deficient (homozygous) transgenic mice. The experiment was reproduced numerous times and analyses of tissues indicated that the protective effect was due to enhanced recovery of hematopoietic cells (bone marrow, spleen, thymus) following irradiation (**FIG. 2**). Injection of chloroquine prior to the TBI had no effect on the survival of mice lacking ATM genes (**FIG. 3**), thus indicating that radioprotection may be dependent on ATM.

[0091] Treatment with chloroquine or hydroxychloroquine also provided significant protection against loss of coat color in surviving mice. **FIG. 10** shows three pairs of mice subject to 8 Gy total body irradiation. The two control mice on the left of the figure show significant loss of coat color. The pair in the middle which were treated with chloroquine before exposure to total body irradiation show no significant loss of coat color. The pair on the right treated with hydroxyquinolone show an intermediate extent of protection.

Example 2

Cancer Prevention

[0092] Transgenic mice expressing the c-myc oncogene under the control of the immunoglobulin enhancer (i.e., Eμ-myc mice) develop B-cell lymphomas and leukemias with relatively short latencies. Chloroquine was added to the drinking water of a cohort of Eμ-myc mice and the mice were observed for the development of B-cell malignancies. **FIG. 4** demonstrates that 100% of the control transgenic mice developed malignancies within 100 days of birth while 0% of the transgenic mice on chloroquine developed tumors. After ~120 days, half of the cohort of chloroquine-treated mice were taken off of chloroquine and the other half were switched to receiving a dose of chloroquine by i.p. injection once a week. Within ~30 days, all of the transgenic mice taken off of the chloroquine had developed tumors while none of the mice receiving weekly i.p. injections developed cancer. At ~10 months of age, these mice on weekly chloroquine remained cancer-free and appeared healthy and normal.

[0093] The carcinogen 3-methylcholanthrene (3-MC) induces soft tissue sarcomas if injected into muscle and skin carcinomas if applied to the skin (Smart, et al. (1986) *Carcinogenesis* 7:1669-1675; Noguchi, et al. (1996) *Proc. Natl. Acad. Sci. U.S.A* 93:11798-11801; Horak, et al. (1984) *Br. J. Cancer* 49:637-644). This model system has been used to demonstrate that superinduction of p53 after DNA damage (e.g., in a mouse carrying an extra copy of chromosomal DNA containing the p53 gene) protects mice from the development of cancers induced by chemical carcinogen treatments (Garcia-Cao, et al. (2002) *EMBO J.* 21:6225-6235). Therefore, it was determined whether the protective effect observed in these studies could likewise be achieved by biochemically enhancing p53 induction. As demonstrated herein, ATM kinase activation by chloroquine did not induce strand breaks or induce phosphorylation of substrates that normally get phosphorylated by ATM at the sites of DNA breaks, however, it did lead to induction and phosphorylation of p53 protein. Thus, chloroquine pre-treatment may prevent/reduce tumor development resulting from 3-MC injections. Accordingly, doses of 3.5 mg/kg of chloroquine were given by i.p. injection 24 and 4 hours prior to 3-MC injection in 30 mice. Results are shown in **FIG. 5**. The occurrence of these tumors was readily apparent by visual inspection and confirmed by histologic assessment.

[0094] In a further experiment, doses of 3.5 mg/kg of chloroquine were given by i.p. injection 24 and 4 hours prior to 3-MC injection in 30 wild type (strain C57Bl/6) mice. The occurrence of these tumors was readily apparent by visual inspection and confirmed by histologic assessment. Results are shown in **FIG. 5**. Treatment with chloroquine significantly increased the percentage of mice surviving tumor free ($p=0.0013$).

[0095] In a further experiment 3-MC was injected into the skin on the leg of a mouse once a week for 4 weeks. Three genetic backgrounds were used: wild-type, ATM-null, and p53-null. One half of each cohort of mice received 3.5 mg/kg of chlorquine (CHQ) via IP injection 24 hours and 4 hours prior to each of the four 3-MC administration. The development of skin carcinomas was followed over time. **FIG. 7** shows tumor incidence in mice receiving either placebo or chloroquine prior to 3-MC injection. Chloroquine markedly protected from tumor development. **FIG. 8** shows tumor incidence in ATM-null mice receiving either placebo or chloroquine prior to 3-MC injection. Chloroquine does not protect from tumor development. **FIG. 9** shows tumor incidence in p53-null mice receiving either placebo or chloroquine prior to 3-MC injection. Again chloroquine did not protect from tumor development. These results showstthat that the prophylactic effect of chloroquine is mediated at least in part through ATM and p53.

[0096] Multiple exposures to non-lethal doses of ionizing radiation can induce thymic lymphomas in C57BL/6 mice (Boniver, et al. (1990) *Int. J. Radiat. Biol.* 57:693-698). Using a classical, tumor-inducing protocol (Kaplan and Brown (1952) *J. Natl. Cancer Inst.* 13:185-208), which consists of four weekly whole-body exposures of 1.75 Gy each, the effect of chloroquine administration on thymic lymphoma formation was examined. Chloroquine (3.5 mg/kg) was administered to 4-week old female C57BL/6 mice by i.p. injection 24 hours and 4 hours prior to each of the four doses of radiation described in the protocol. According to the protocol, tumors were expected to appear within 4-6 months after the last dose of irradiation in 90% of control (untreated) mice. **FIG. 6** shows the results of this analysis.

[0097] All patents, publications, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual patent, publication, or patent application was specifically and individually indicated to be incorporated by reference.

[0098] It will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

1. A method of treatment for a radiation related disorder comprising administering to an animal subject in need thereof an effective amount of a chloroquine compound.

2. The method of claim 1 wherein said radiation related disorder is caused by an exposure to a non-ionizing radiation.

3. The method of claim 1 wherein said radiation related disorder is caused by an exposure to an ionizing radiation.

4. The method of claim 3 wherein said chloroquine compound treats a skin damage symptom caused by said ionizing radiation.

5. The method of claim 4 wherein said skin damage symptom is at least one symptom selected from swelling, itching, redness of skin, and hair loss.

6. The method of claim 3 wherein said chloroquine compound treats a non-skin damage symptom caused by said ionizing radiation.

7. The method of claim 6 wherein said non-skin damage symptom is at least one symptom selected from nausea, vomiting, diarrhea, loss of appetite, fatigue, fever, diminished organ function, seizures, cancer, and coma.

8. The method of claim 1 wherein said radiation related disorder is acute radiation syndrome.

9. The method of claim 1 wherein said chloroquine compound prevents a death due to radiation exposure.

10. The method of claim 1 wherein said chloroquine compound protects from sub-lethal and/or unintentional radiation exposure.

11. The method of claim 1 wherein said chloroquine compound protects from sub-lethal and/or lethal intentional radiation exposure.

12. The method of claim 11 wherein said intentional radiation exposure is due to an act of terrorism.

13. A method of treatment for an adverse effect of a radiation therapy comprising administering to an animal subject in need thereof an effective amount of a chloroquine compound.

14. The method of claim 13 wherein said adverse effect is at least one effect selected from a skin damage, alopecia, or neutropenia.

15. The method of claim 1 or **13** wherein said chloroquine compound is at least one compound selected from chloroquine, chloroquine phosphate, hydroxychloroquine, chloroquine diphosphate, chloroquine sulphate, hydroxychloroquine sulphate, or enantiomers, derivatives, analogs, metabolites, pharmaceutically acceptable salts, and mixtures thereof.

16. The method of claim 15 wherein said compound is at least one compound selected from chloroquine, chloroquine phosphate, hydroxychloroquine, chloroquine diphosphate.

17. The method of claim 16 wherein said compound is chloroquine.

18. The method of claim 16 wherein said compound is hydroxychloroquine.

19. The method of claim 17 or **18** wherein said compound is an essentially pure (+) isomer.

20. The method of claim 17 or **18** wherein said compound is an essentially pure (−) isomer.

21. The method of claim 1 or **13** wherein the amount of the compound administered is at least about 0.1 mg/kg/day.

22. The method of claim 1 or **13** wherein the amount of the compound administered is up to about 10 mg/kg/day.

23. The method of claim 1 or **13** wherein the amount of the compound administered is more than about 0.1 mg/kg/day.

24 The method of claim 1 or **13** wherein the amount of the compound administered is more than about 1.0 mg/kg/day.

25. The method of claim 1 or **13** wherein the amount of the compound administered is less than about 50 mg/kg/day.

26. The method of claim 1 or **13** wherein the amount of the compound administered is less than about 10 mg/kg/day.

27. The method of claim 1 or **13** wherein the chloroquine compound is administered more than once a week.

28. The method claim 1 or **13** wherein the chloroquine compound is administered daily, every two weeks, or once a month.

29. The method of claim 1 or **13** wherein the chloroquine compound is formulated in a sustained release formulation.

30. The method of claim 1 or **13** wherein said amount of said compound administered is about 0.1 to about 9 mg/kg once a week.

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