(54) Title: USE OF METALLOPORPHYRINS TO REVERSE THE TOXIC EFFECT OF TUMOR THERAPY

(57) Abstract

Treatment with metalloporphyrins of patients undergoing tumor therapy particularly chemotherapy with anthraacycline type agents reverses the toxic effects of such therapy.
<table>
<thead>
<tr>
<th>AT</th>
<th>Austria</th>
<th>FR</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>KR</td>
<td>Republic of Korea</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>DE</td>
<td>Germany, Federal Republic of</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>FI</td>
<td>Finland</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

ML  | Mali           |
MR  | Mauritania     |
MW  | Malawi         |
NL  | Netherlands    |
NO  | Norway         |
RO  | Romania        |
SD  | Sudan          |
SE  | Sweden         |
SN  | Senegal        |
SU  | Soviet Union   |
TD  | Chad           |
TG  | Togo           |
US  | United States of America |
USE OF METALLOPORPHYRINS TO REVERSE THE TOXIC EFFECT OF TUMOR THERAPY

BACKGROUND OF THE INVENTION

The synthetic heme analog tin protoporphyrin-IX (SnPP) is a potent inhibitor of heme oxygenase, the rate limiting enzyme in the degradation of heme to bile pigment. In addition SnPP is also known as a complete suppressant of hyperbilirubinemia in neonatal mammals and for its ability to reduce plasma bilirubin levels in a variety of forms of naturally occurring or experimentally induced jaundice in animals and man.

Two analogs of SnPP are also known for the same activity. These are tin mesoporphyrin (SnMP) and tin diiododeuteroporphyrin (SnI₂DP).

It is known that the presence of tumors, whether spontaneous or implanted increases heme oxygenase activity, the rate limiting enzyme of heme catabolism, decreases aminolevulinate synthetase (ALA synthetase) activity, the rate limiting enzyme of heme synthesis, the concentration of microsomal heme, the concentration of cytochrome P-450 in liver and the activities of mixed function oxidases dependent on this heme protein. Various types of treatments for cancer, especially chemotherapy are known to increase these effects.
A large number of antineoplastic chemotherapeutic agents are known. Their activities are based on a variety of metabolic activities. These include alkylating agents such as N-alkyl-N-nitrosoureas and mitomycin-C, antimetabolites such as 6-mercaptopurine and 5-fluorouracil, inhibitors of protein synthesis such as anquidine and trichodermol, and anthracycline type antitumor agents which function by intercalation into DNA as well as alkylation, inhibition of protein synthesis, generation of free radicals and depletion of hepatic (and cardiac) glutathione stores. Intercalation is a process by which the antitumor agent moves into a separation between adjacent base pairs in the DNA helix causing profound changes in the properties of the intercalated DNA. Anthracycline type antineoplastic agents are, at this stage in the art, by far the most widely employed therapeutically useful products of this type. This class of antitumor antibiotic is clinically active in human leukemia, lymphoma (Hodgkins and Non-Hodgkins), breast, germ cell, lung (small cell), sarcoma, gastric and ovarian cancers.

The anthracyclines are a well known class of antitumor agents that have been employed in the clinical treatment of various tumors in humans since at least 1970. The best known of the class is doxorubicin. It has a wide spectrum of antitumor activity, such activity encompassing a broad range of solid tumors that prior to its isolation from Streptomyces peucetius var caesius had been relatively insensitive to chemotherapy, especially the soft tissue and bone sarcomas and bladder cancer. These activities of doxorubicin and other members of the class against various tumors have been discussed extensively by Carter in the Journal Of The National Cancer Institute, Vol. 55, No. 6, December 1975, pages 1265 to 1274.

SUBSTITUTE SHEET
Other anthracyclines, which have received clinical attention include daunorubicin, carminomycin and AD-32. Although there are other promising candidates, only doxorubicin and daunorubicin have, thus far, achieved widespread clinical acceptance.

The anthracyclines have been divided into two classes based on their selective effects on the inhibition of nucleic acid synthesis. Type I anthracyclines, exemplified by doxorubicin, daunorubicin and carminomycin inhibit DNA, whole cell RNA, and nucleolar RNA synthesis at approximately comparable concentrations, whereas Type II anthracyclines, exemplified by mytomycin-C aclacinomycin and marcellomycin inhibit whole cellular RNA synthesis at six- or seven-fold lower concentrations and nucleolar preribosomal synthesis at 170-1250-fold lower concentrations than those required for the inhibition of DNA synthesis. Both types have equivalent toxic effects. This invention is applicable to both types.

The anthracyclines are metabolized by cytochrome P-450 in the liver. Thus it is essential that normal levels of this heme protein be maintained in the liver.

THE INVENTION

It has been discovered that treatment of tumor bearing mammals with SnPP, SnMP or SnI₂DP decreases heme oxygenase activity and normalizes ALA synthetase activity, hepatic heme and cytochrome P-450 levels, thus reversing the toxic effects of cancer therapy or tumor burden. It has further been discovered that parenteral coadministration of these metalloporphyrins together with chemotherapeutic agents,
especially anthracycline type antitumor agents such as
doxorubicin will reverse the toxic effects of the
chemotherapeutic agent on heme and cytochrome
P-450. Coadministration of the metalloporphyrics and the
chemotherapeutic agent means that the products are
administered within a time frame in which the metallic
porphyrics will exert their beneficial effects. This can
mean several days, e.g. two to five days before initiation of
chemotherapy. It can mean, and in preferred instances will
mean, that the two agents are administered together,
preferably in the same dosage unit.

Typically, chemotherapeutic treatment with agents such as
doxorubicin follows an intense regimen in which the highest
tolerable dosage is administered and continued until the
toxic effects become so severe that it is necessary to
decrease the dosage level or withhold treatment until the
toxicity effects are substantially neutralized. At this
point the cycle is repeated. The principal advantage of this
invention is that it permits the use of the selected
chemotherapeutic at higher levels for longer periods of time
by maintaining normal levels of cytochrome P-450 and other
related enzymes in the body tissues.

This invention, as will be apparent from the foregoing
discussion comprises, in its broadest sense, a method of
preventing the decline in heme and cytochrome P-450 in tumor
bearing mammals, whether or not such mammals are undergoing
tumor therapy, by administration to a tumor bearing patient
in need of such treatment either because of an untreated
tumor burden or because the mammal is undergoing anti-
neoplastic therapy, an amount of SnPP, SnMP or SnI$_2$DP which
is effective to achieve the desired result. More
specifically, it comprises a method of reversing the toxic
effects caused by the presence of tumor burden whether or not there is chemotherapy, by administration to a patient a therapeutically effective amount of SnPP, SnMP or SnI₂DP to cause the reversal of loss of hepatic heme and cytochrome associated with the tumor whether or not the patient is undergoing chemotherapy. The invention also includes therapeutic compositions for use in the practice of the methods.

The efficacy of the products of the invention for their newly discovered utility was established by experiments with three groups of male Sprague-Dawley rats (males, 150-200 grams, 16 per group). The rats were maintained on ad lib water and powdered rat chow (Purina) during the experiments. Group A was untreated, Group B was treated subcutaneously with doxorubicin alone on day 0, and Group C was treated with SnPP one day prior to doxorubicin which was administered on day 0.

The effects of doxorubicin (10 mg/kg b.w.) on heme metabolism were studied with time and are shown in the figure. It will be seen that the increase in heme oxygenase activity and decrease in ALA synthetase activity and microsomal content of cytochrome P-450 were at a maximum on day 2 and returned to normal by day 10. Substantially the same results were observed at a doxorubicin dosage level of 5 mg/kg b.w.

As shown in Table 1, subcutaneous treatment of the rodents with 10 mg/kg b.w. doxorubicin produced a statistically significant (P < 0.05) increase in hepatic heme oxygenase activity (20%) and a concomitant decrease in ALA synthetase activity (58%), and in the microsomal content of heme (45%) and cytochrome P-450 (18%) when these parameters were...
measured 2 days after doxorubicin administration. SnPP, when administered one day prior to doxorubicin, prevented the occurrence of these variations in heme metabolism. The group treated with doxorubicin and SnPP demonstrated a significant decrease (92%) in hepatic heme oxygenase activity and a significant increase (35%) in hepatic ALA-synthetase activity when compared to the group treated with doxorubicin alone. The levels of hepatic cytochrome P-450, and microsomal heme were substantially identical to those in the untreated group indicating that SnPP treatment prevented the detrimental effects of doxorubicin on heme metabolism. Similar effects are achieved with SnMP and SnI₂PP and with other anthracycline type antitumor agents.
<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>Iodophene Treatment</th>
<th>p-420 Control</th>
<th>p-420 Iodophene Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heme Synthase</td>
<td>1.39 ± 0.04</td>
<td>1.30 ± 0.03*</td>
<td>0.71 ± 0.05</td>
<td>0.68 ± 0.08*</td>
</tr>
<tr>
<td>ALA Synthase</td>
<td>0.77 ± 0.01</td>
<td>0.75 ± 0.01</td>
<td>0.49 ± 0.00</td>
<td>0.49 ± 0.00</td>
</tr>
<tr>
<td>Heme Oxygenase</td>
<td>3.04 ± 0.23</td>
<td>3.04 ± 0.23*</td>
<td>2.30 ± 0.11</td>
<td>2.30 ± 0.11*</td>
</tr>
<tr>
<td>Formed/hr/mg protein</td>
<td>0.17 ± 0.01</td>
<td>0.17 ± 0.01*</td>
<td>0.17 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Formed/hr/mg protein</td>
<td>0.30 ± 0.01</td>
<td>0.30 ± 0.01*</td>
<td>0.30 ± 0.01</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>Formed/hr/mg protein</td>
<td>0.65 ± 0.02</td>
<td>0.65 ± 0.02*</td>
<td>0.65 ± 0.02</td>
<td>0.65 ± 0.02</td>
</tr>
</tbody>
</table>

*: statistically significant difference from control (p < 0.05).
The animals utilized in the tests were acclimated in metabolic cages maintained in a controlled environment with a 12h/12h light/dark cycle.

The compositions for parenteral administration were freshly prepared for each injection by addition of 0.4 N NaOH (20% v/v) to prepare a solution at pH 12, adjusted to pH 7.5 with 1 NHCl and made to final volume with 0.9% saline. The dosage level per injection was 50 umol/kg b.w. Control animals were administered an equivalent amount of saline.

Animals were deprived of food but allowed free access to water for 16 hours prior to sacrifice. Livers were perfused in situ with ice-cold saline and homogenized in tris-HCL (0.01 M, pH 7.4) buffer containing sucrose (0.25 M). Homogenates (25% w/v) were centrifuged at 9000 g for 20 min, and the resulting mitochondrial pellet was used for the assay of ALA-synthetase activity. The 9000 xg supernatant was centrifuged at 105,000 xg for 60 min. in a Beckman L5-50 ultracentrifuge. The microsomal pellet was resuspended in a minimum volume of potassium phosphate buffer (0.1 M, pH 7.4) and the cytosol was used as a source of biliverdin reductase. Cytochrome P-450 content was determined by the methods of Omura and Sato [J. Biol. Chem., 239: 2379 (1966) and 239: 2370 (1966)], and protein concentration by the method of Lowry et al [J. Biol. Chem., 193 265 (1951)] using crystalline bovine serum albumin as standard. Heme, ALA synthetase and heme oxygenase were assayed as described by Drummond and Kappas PNAS, 78, 6466-6470 (1981). Statistics were performed by Student's t-test (Prophet).
The products of the invention will be provided as parenteral compositions for injection. The selected metallic porphyrin or porphyrins can be suspended in an inert oil such as sesame, peanut or olive oil. Alternatively they can be suspended in an aqueous isotonic buffer at a pH of about 7 to 8, preferably 7.4 to 7.5. Useful buffers include sodium citrate-citric acid and sodium phosphate-phosphoric acid.

The desired isotonicity may be accomplished with sodium chloride or other pharmaceutically acceptable agents such as glucose, boric acid, sodium tartrate, propylene glycol or other organic or inorganic solutes. Sodium chloride and glucose are preferred because they are readily and inexpensively available in pure form.

The compositions, which may contain a metalloporphyrin as the principal active ingredient or a mixture of metalloporphyrin and chemotherapeutic agent such as doxorubicin, can also be prepared in lyophilized form for reconstitution in an aqueous medium such as isotonic saline or an isotonic buffer.

The attending physician will determine the optimum dose based on factors such as the selected metalloporphyrin, the age, weight, general health, tumor burden and other factors well known to those skilled in the art. The compositions will normally be provided in dosage unit form for one or a plurality of treatments. A dosage unit form for parenteral administration will typically contain from 7.15 to 71.5 mg/ml SnPP; 0.719 to 7.19 mg/ml SnMP; or 9.15 to 91.5 mg/ml SnI$_2$DP so as to provide dosages of from 0.715 to 7.15 mg/kg b.w.; 0.0719 to 7.19 mg/kg b.w.; and 0.915 to 9.15 mg/kg b.w. respectively. The amount of chemotherapeutic agent per dosage unit will, of course, vary with the selected agent,
the clinical state of the patient and other factors which the physician in attendance can evaluate. For doxorubicin, each dosage unit as prepared for injection will contain about 20 to 70 mg/ml doxorubicin, typically as a pharmaceutically acceptable acid salt.

A dosage unit may also be provided as a container holding sufficient lyophilized powder to be reconstituted in an aqueous medium to contain the desired amounts of metalloporphyrin and, if desired, antineoplastic agent.

The therapeutic compositions of this invention will be prepared by the usual procedures employed for such purposes and need not be described here.
WHAT IS CLAIMED IS

1. A method of reversing the toxic effects of tumor therapy by administration to a patient undergoing such therapy and in need of such reversal, an amount of SnPP, SnMP or SnI₂DP which is effective to cause such reversal.

2. A method of reversing the toxic effects of chemotherapy by coadministration to a patient undergoing such therapy, and in need of such reversal, a therapeutically effective amount of a chemotherapeutic agent together with an amount of SnPP, SnMP or SnI₂DP which is effective to cause such reversal.

3. A method as in claim 2 wherein the chemotherapeutic agent is an anthracycline type antitumor agent.

4. A method as in claim 3 wherein the anthracycline type antitumor agent is doxorubicin.

5. A pharmaceutical composition for parenteral administration comprising a pharmaceutically acceptable carrier and, as the principal active ingredients SnPP, SnMP or SnI₂DP together with a chemotherapeutic agent.

6. A pharmaceutical composition as in claim 5 wherein the chemotherapeutic agent is an anthracycline type antitumor agent.

7. A pharmaceutical composition as in claim 6 wherein the anthracycline type antitumor agent is doxorubicin.
8. A pharmaceutical composition as in claims 5, 6 or 7 wherein the pharmaceutical carrier is buffered isotonic aqueous saline solution.

9. A pharmaceutical composition as in claims 5, 6 or 7 wherein the pharmaceutical carrier is buffered isotonic aqueous glucose solution.

10. A lyophilized composition comprising a chemotherapeutic agent and SnPP, SnMP or SnI₂PP.

11. A lyophilized composition as in claim 10 wherein the chemotherapeutic agent is an anthracycline type antitumor agent.

12. A lyophilized composition as in claim 11 wherein the anthracycline type antitumor agent is doxorubicin.
○ - ALA-S Activity

▲ - Cytochrome P-450 Content

○ - Heme Oxygenase Activity
**INTERNATIONAL SEARCH REPORT**

**I. CLASSIFICATION OF SUBJECT MATTER**

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 31/555
U.S.Cl.: 424/10; 514/85,922

**II. FIELDS SEARCHED**

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Classification Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.</td>
<td>514/185,922; 424/10</td>
</tr>
</tbody>
</table>

Documentation Searched other than Minimum Documentation to the extent that such documents are included in the fields searched.

**III. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, 11 with indication, where appropriate, of the relevant passages 12</th>
<th>Relevant to Claim No. 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Chemical Abstracts, Volume 94, No. 25, Issued 22 June 1981 (Columbus, Ohio, USA, Marchand et al., &quot;Depression of Cytochrome P-450-dependent drug Biotransformation by Adriamycin&quot;, see page 38, column 2, the Abstract No. 94:202626k, Toxicol. Appl. Pharmacol. 1981, 58(1), 83-8 (Eng)).</td>
<td>1-4</td>
</tr>
<tr>
<td>Y</td>
<td>Chemical Abstracts, Volume 93, No. 25, Issued 22 December 1980 (Columbus, Ohio USA, Ehninger et al., &quot;Pharmacokinetics of adriamycin and adriamycin metabolites&quot;, see page 20, column 1, the Abstract No. 93:230559p, Klin. Wochenschr. 1980, 58(18), 927-34 (Ger)).</td>
<td>1-4</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:
  - "E" earlier document but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed
  - "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
  - "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
  - "A" document member of the same patent family

**IV. CERTIFICATION**

Date of the Actual Completion of the International Search: 24 OCTOBER 1988
Date of Mailing of this International Search Report: 05 DEC 1988

International Searching Authority: ISA/US
Signature of Authorized Officer: Richard Kearse
Richard Kearse
FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

| Y | Chemical Abstracts, Volume 101, No. 25.  
   | Issued 17 December 1984 (Columbus, Ohio, USA, Kappas et al., "Control of heme and cytochrome P450 metabolism by inorganic metals, organometals, and synthetic metallocorphyrins", see page 220, column 2, the Abstract No. 101:224234w, EPH, Environ. Health Perspect 1984. 57, 301-6 (Eng)). | 1-4 |

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 19(2) (a) for the following reasons:

1. Claim numbers ________, because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim numbers ________, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claim numbers ________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invoice payment of any additional fee.

Remark on Protest:

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>U.S. A, 4,668,670 (RIDEOUT ET AL) 26 May 1987, see column 1, lines 23-66; column 6, lines 36-38 and 53-59.</td>
<td>1-4</td>
</tr>
<tr>
<td>Y</td>
<td>U.S. A, 4,386 087 (LAVALLEE) 31 May 1983, see column 1, lines 64-65; column 3, lines 21-26.</td>
<td>5-12</td>
</tr>
<tr>
<td>Y</td>
<td>U.S. A, 4,692,439 (RIDEOUT ET AL) 8 September 1987, see column 2, lines 58-68; column 5. lines 41-65; column 6 lines 55-66; column 8, lines 16-30.</td>
<td>5-12</td>
</tr>
</tbody>
</table>