United States Patent [19]

Surendra et al.

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[54] **USE OF RAPAMYCIN IN TREATMENT OF CERTAIN TUMORS**

[75] Inventors: **Sehgal N. Surendra,** Dollard Des Ormeaux; **Claude Vezina,** Oka, both of Canada

[73] Assignee: **American Home Products Corporation,** New York, N.Y.

[21] Appl. No.: **592,193**

[22] Filed: **Mar. 22, 1984**

Related U.S. Application Data

[63] Continuation of Ser. No. 126,276, Mar. 3, 1980, abandoned, which is a continuation of Ser. No. 957,626, Nov. 3, 1978, abandoned.

[51] **Int. Cl.**[4] **A61K 35/74**

[52] **U.S. Cl.** **424/122**

[58] **Field of Search** 424/122

[56] **References Cited**

PUBLICATIONS

Endicott, J. of the National Cancer Institute, The Chemotherapy Program, vol. 19, No. 2 (20th Anniversary) Aug. 1957, pp. 275–293.

Primary Examiner—Jerome D. Goldberg
Attorney, Agent, or Firm—Walter Patton

[57] **ABSTRACT**

Methods for using rapamycin in the treatment of certain cancers or tumors are disclosed.

7 Claims, No Drawings

USE OF RAPAMYCIN IN TREATMENT OF CERTAIN TUMORS

This is a continuation of application Ser. No. 126,276, filed Mar. 3, 1980 which in turn is a continuation of application Ser. No. 957,626, filed Nov. 3, 1978, both now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the use of rapamycin as an anti-cancer or anti-tumor agent.

2. Description of the Prior Art

Rapamycin is an antifungal antibiotic described by C. Vezina et al., J. Antibiot., 28, 721 (1975), S. N. Sehgal et al., J. Antibiot., 28, 727 (1975) and S. N. Sehgal et al., U.S. Pat. No. 3,929,992, issued Dec. 30, 1975, filed Apr. 12, 1974. Rapamycin is extracted from a streptomycete (*Streptomyces hygroscopicus*) isolated from an Easter Island soil sample and is particularly effective against *Candida albicans* both in vitro and in vivo.

In addition, a recent report by R. R. Martel et al., Can. J. Physiol., 55, 48 (1977) describes the use of rapamycin for the prevention of the development of two experimental immunopathies [(experimental allergic encephalomyelitis (EAE) and adjuvant arthritis (AA)]. The latter report also describes the inhibitory effect of rapamycin on the formation of humoral (IgE-like) antibody. This report concludes that immunosuppressant activity of rapamycin appears to be related to inhibition of the lymphatic system.

SUMMARY OF THE INVENTION

According to this invention a method is provided for treating carcinogenic tumors in a mammal which comprises administering to the mammal an antitumor effective amount of rapamycin. More specifically, rapamycin reduces tumor size in and prolongs the survival time of tumor bearing mammals.

DETAILS OF THE INVENTION

According to the present method, rapamycin is employed as the active agent. The isolation and description of rapamycin is given in U.S. Pat. No. 3,929,992, cited above, herein incorporated by reference.

Rapamycin is administered to a carcinogenic tumor bearing mammal for the purpose of reducing the tumor size and prolonging the survival time of the tumor bearing mammal, either orally or parenterally.

While rapamycin can be administered above, e.g. as a sole component of a filled capsule, it is preferred to formulate the compound in various dosage forms for oral or parenteral administration, e.g. tablets or sterile solutions. Such formulations are described in U.S. Pat. No. 3,929,992, cited above.

When utilizing rapamycin for the treatment of tumors, the total dose of active agent can range from 0.5 to 500 mg per kg of body weight per day with a preferred dosage range from 10 to 250 mg per kg of body weight per day. However as the dosage of rapamycin to be administered by the method of this invention will of course vary with the tumor or cancer and tolerance of the mammal, it is preferred to initiate treatment of the tumor bearing mammal with a low daily dose of rapamycin and then to gradually increase the dosage until a desirable reduction in tumor size is achieved without causing any harmful or deleterious side effects. The schedule of dosing can range from one to five times per day to a single dose given every two to ten days. Such dosages and scheduling of administration must be determined on an individual basis, depending upon the tumor or cancer, nutritional state of the mammal, age of the mammal, toxicity in each individual, etc.

Rapamycin reduces tumor size in and prolongs the survival time of tumor-bearing mammals. More specifically, rapamycin is useful for controlling the following carcinogenic tumors in a mammal: lymphatic leukemia, colon, mammary, melanocarcinoma and ependymoblastoma. The effectiveness of rapamycin in this respect can be demonstrated in the laboratory with rodents having transplanted tumors. Details of methods used to evaluate this effect are described in various publications; for example, R. I. Geran et al., Cancer Chemother. Rep., Part 3, 3, (No. 2) 1-103 (1972) and references therein. In addition the protocols for the antitumor tests are available from the National Cancer Institute, Bethesda, Md., U.S.A.

Tables 1 to 6 show the effects of therapy with rapamycin on various tumor or cancers in rodents.

More specifically, Table 1 shows the prolongation of survival time of female CDF_1 mice implanted with lymphatic leukemia P338 by administering rapamycin; Table 2 shows the reduction in size of colon 38 tumors in female BDF_1 mice by administering rapamycin; Table 3 shows the prolongation of survival time of male CDF_1 mice implanted with colon 26 tumors by administering rapamycin; Table 4 shows the reduction in size of $CD8F_1$ mammary tumors in male CD8F1 rats by administering rapamycin; Table 5 shows the prolongation of survival time of female BDF_1 mice implanted with B16 melonocarcinoma by administering rapamycin; and Table 6 shows the prolongation of survival time of male Swiss mice implanted with ependymoblastoma by administering rapamycin.

TABLE 1

Effect of Rapamycin on Survival Time CDF_1 Mice Implanted with Lymphatic Leukemia P-388(ascetic).

Dose/Inj mg/kg	Ave. Wt. Difference of Animals (T-C, g)	Survivors on Day 5	MST days T	MST days C	T/C % MST
400	−1.9	6/6	14.1	10.2	138
200	−2.4	6/6	13.1	10.2	128
100	−1.6	6/6	13.7	10.2	134
50	−1.9	6/6	14.3	10.2	140
25	−1.6	6/6	13.9	10.2	136
12.5	−0.6	6/6	13.9	10.2	136

Treatment:
Nine intraperitoneal injections starting on day one in a vehicle of saline with Tween-80 [Trade Mark for a derivative of Z-sorbitan mono-9-octadecenoate poly-(oxy-1,2-ethanediyl)].
Evaluation:
T/C % = Median Survival Time (MST) in days of treated animals (T) control animals (c) × 100. A T/C % of 125 or greater is considered as a significant prolongation of host survival. Evaluation done on day 30.

TABLE 2

Effect of Rapamycin on Colon 38 Tumor Weight in Mice

Dose/Inj mg/kg	Ave. Net Wt. Difference of Animals (T-C, g)	Survivors Day 5	MTW mg T	MTW mg C	T/C % MTW
400	−3.4	10/10	188	810	23
200	−2.0	10/10	209	810	25
100	−0.8	10/10	272	810	33
50	−0.8	9/10	320	810	39
25	−0.4	10/10	368	810	45

TABLE 2-continued

Effect of Rapamycin on Colon 38 Tumor Weight in Mice

Dose/Inj mg/kg	Ave. Net Wt. Difference of Animals (T-C, g)	Survivors Day 5	MTW mg T	MTW mg C	T/C % MTW
12.5	0.4	10/10	368	810	45

Treatment:
Single intraperitoneal injection on days 2, 9 and 16 in a vehicle of saline with Tween-80.
Evaluation:
T/C % = Median tumor weight (MTW) estimated from tumor diameter of treated animals (T)/control animals (C) × 100. A T/C % of 42 or less is considered as a significant inhibitor of tomor growth. Evaluation done on day 20.

TABLE 3.

Effect of Rapamycin on Survival Time of CDF_1 Mice Implanted with Colon 26 Tumor

Dose/Inj. mg/kg	Ave. Wt. Difference of Animals (T-C, g)	Survivors on Day 5	MST days T	MST days C	T/C % MST
400	−2.4	10/10	26.3	19.1	137
200	−1.8	10/10	25.8	19.1	135
100	−1.4	10/10	29.0	19.1	151
50	−0.8	10/10	30.6	19.1	160
25	−0.3	10/10	30.3	19.1	158
12.5	0.3	10/10	30.4	19.1	159

Treatment:
Single intraperitoneal injection on days 1, 5 and 9 in a vehicle of saline with Tween-80.
Evaluation:
T/C % = Median Survival Time (MST) in days of treated animals (T) control animals (C) × 100. A T/C % of 125 or greater is considered as a significant prolongation of host survival. Evaluation done on day 60.

TABLE 4

Effect of Rapamycin on $CD8F_1$ Mammary Tumors in $CD8F_1$ Rats

Dose/Inj mg/kg	Ave. Net Wt. Difference of Animals (T-C, g)	Survivors Day 5	MTW mg T	MTW mg C	T/C % MTW
400	−6.6	4/10	0	3200	—
200	−6.5	10/10	323	3200	10
100	−4.8	10/10	448	3200	14
50	−4.1	10/10	755	3200	23
25	−2.4	10/10	825	3200	25
12.5	−0.8	10/10	928	3200	29

Treatment:
Single intraperitoneal injection on days 1, 8, 15, 22 and 29 in vehicle of saline with Tween-80.
Evaluation:
T/C % = Median tumor weight (MTW) estimated from tumor diameter of treated animals (T)/control animals (C) × 100. A T/C% of 42 or less is considered as a significant inhibitor of growth. Evaluation done on day 30.

TABLE 5

Effect of Rapamycin on B 16 melanocarcinoma in BDF_1 Mice

Dose/Inj. mg/kg	Ave. Wt. Difference of Animals (T-C, g)	Survivors on Day 5	MST days T	MST days C	T/C % MST
400	−3.3	10/10	22.0	20.1	109
200	−1.5	10/10	22.3	20.1	110
100	−1.2	10/10	28.0	20.1	139
50	−0.7	10/10	25.3	20.1	125
25	0.1	10/10	28.0	20.1	139
12.5	0.1	10/10	29.0	20.1	144

Treatment:
Single intraperitoneal injection on each of days 1 through 9 in a vehicle of saline with Tween-80.
Evaluation:
T/C % = Median Survival Time (MST) in days of treated animals (T) control animals (C) × 100. A T/C % of 125 or greater is considered as a significant prolongation of host survival. Evaluation done on day 60.

TABLE 6

Effect of Rapamycin on Ependymoblastoma in Swiss Mice

Dose/Inj. mg/kg	Ave. Wt. Difference of Animals (T-C, g)	Survivors on day 5	MST days T	MST days C	T/C % MST
200	−3.3	10/10	44.0	18.1	243
100	−2.2	10/10	26.0	18.1	143
50	−1.3	9/10	34.0	18.1	187
25	−2.0	10/10	34.0	18.1	187
12.5	−1.0	10/10	32.3	18.1	178

Treatment:
Single intraperitoneal injection on each of days 1 through 9 in a vehicle of saline with Tween-80.
Evaluation:
T/C % = Median Survival Time (MST) in days of treated animals (T) control animals (C) × 100. A T/C % of 125 or greater is considered as a significant prolongation of host survival. Evaluation done on day 60.

Rapamycin also can be used to produce beneficial effects in the treatment of malignant tumors when combined with a therapeutically effective amount of an antineoplastic agent commonly used in cancer therapy. Such antineoplastic agents include the alkylating agents, for example, busulfan, chlorambucil, cyclophosphamide, mechlorethamine hydrochloride, melphalan, pipobroman, thiotepa and uracil mustard; antimetabolites, for example, cytarabine, fluorouracil, floxuridine, mercaptopurine, methotrexate and thioguanine; miscellaneous anticancer agents, for example, dacarbazine, hydroxyurea, mitotane, procarbazine hydrochloride, quinacrine hydrochloride, vinblastine sulfate and vincristine sulfate; estrogens, for example, chlorotrianisene, conjugate estrogens (e.g. PREMARIN ®), diethylstilbestrol and the like; androgens, for example, methyltestosterone, testosterone and the like; adrenal corticosteroids, for example, prednisone and the like; progestagens, for example, megestrol, hydroxyprogesterone caproate and the like; radioactive isotopes; and antibiotics, for example, bleomycin sulfate, doxorubicin hydrochloride and the like. Suitable methods of administration, compositions and dosages of the antineoplastic agents are described in medical textbooks; for instance, "PHYSICIANS' DESK REFERENCE", 32nd ed., Medical Economics Co., Oradell, N.J. U.S.A., 1978 and "AMA DRUG EVALUATIONS", 3 ed. PSG Publishing Company, Inc., Littleton, Mass., U.S.A., pp 1106–1151, 1977. When used in combination, rapamycin is administered as described previously; however, a lower dose can be used for efficacious results.

We claim:

1. A method of treating transplanted tumors in a transplanted tumor bearing mammal, wherein said tumor is selected from lymphatic leukemia, colon, mammary, melanocarcinoma and ependymoblastoma tumors which comprises administering to said mammal an anti-tumor effective amount of rapamycin.

2. The method of claim 1 wherein rapamycin is administered orally or parenterally.

3. The method of claim 2 wherein rapamycin is administered intraperitoneally as a solution in saline with a derivative of (Z)-sorbitan mono-9-octadecenoate poly-(oxy-1,2-ethanediyl).

4. The method of claim 2 wherein rapamycin is administered at a daily dose of 0.5 to 500 mg per kg of body weight.

5. The method of claim 2 wherein rapamycin is administered at a daily dose of 10 to 250 mg per kg of body weight.

6. The method of reducing tumor size in a colon tumor bearing mammal, comprising administering to said mammal an anti-colon tumor effective amount of rapamycin.

7. The method of prolonging the survival time of a colon tumor bearing mammal, which comprises administering to said mammal an anti-colon tumor effective amount of rapamycin.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,885,171

DATED : December 5, 1989

INVENTOR(S) : Surendra N. Sehgal et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page; Item [19] Sehgal et al.

[75] Inventors: Surendra N. Sehgal, Dollard Des Orneaux; Claude Vezina, Oka, both of Canada

[73] Assignee: Ayerst, McKenna & Harrison, Inc. Ville St. Laurent, Quebec, Canada

Signed and Sealed this

Twenty-sixth Day of January, 1993

Attest:

STEPHEN G. KUNIN

Attesting Officer

Acting Commissioner of Patents and Trademarks