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(54) Title: PRODUCT AND METHOD FOR KILLING ABNORMAL VERTEBRATE CELLS

(57) Abstract

A product and method for killing undesirable abnormal tissue or blood cells while saving normal tissues or cells. A diaminoacridine compound, proflavine in the preferred embodiment, lyses abnormal cells over a period of 6 hours to 2 days when those cells are exposed to diaminoacridine over that period of time at concentrations of at least 1 mg/kg of body weight. Concentrations of diaminoacridines of at least 15 mg/kg of body weight are harmless to humans beyond causing a transitory hypersensitivity to sun light. Specifically, proflavine is a useful treatment for cancer and, in theory, should be useful against AIDS, rheumatoid arthritis and psoriasis.

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PRODUCT AND METHOD FOR KILLING ABNORMAL VERTEBRATE CELLS

Background Of The Invention

Field Of The Invention

The present invention relates to a compound and method for killing undesirable abnormal tissue or blood cells while saving normal tissue or blood cells from damage by means of the administration of diaminoacridines.

Description Of The Prior Art

It is well known that existing anti-tumor chemotherapies have not, in general, been as selective as anti-microbial chemotherapies. Prior to the present invention, most investigators found a direct correlation between cytotoxicity in vitro and anti-tumor activity in vivo, though without selective toxicity for tumor cells as compared with normal cells. Thus the goal of chemotherapeutic research has been to find a therapy which works selectively against tumor cells or abnormal cells and which therapy is not toxic to normal cells. Prior to the present investigation, this search has not been successful.

Diaminoacridines such as acriflavine and proflavine were used as systemic anti-gonorrheal agents in the 1910's and 1920's. They were usually administered intravenously. Bohland in 1919 found that 200 mg. of proflavine could be injected daily into 140 pound adults without injury. Animal studies done by Browning indicate that rabbits could be given an intravenous injection of up to 70 mg. per kg. of

body weight without damage. After sacrificing the animals, Browning found that all tissues, with the exception of nervous tissue, were stained. Browning found that the pharmacological activity of proflavine sulfate was not adversely affected by serum. Zeleney and Mau in 1928 summarized the belief that has prevailed up to the present invention that the systemic intravenous injection of acriflavine and other diaminoacridines is never justified because the beneficial effect is so small relative to the toxicity to the organism. This statement is true as far as the anti-microbial effect of the diaminoacridines is concerned. It is this fact which, in the inventor's opinion, has led to the beneficial tumoricidal effects of systemic injections of diaminoacridines being overlooked.

Though some investigators, most notably Lewin and Blumenthal, reported that trypaflavin and argoflavin injected intravenously into human cancer patients effected an improvement, most studies indicate, and the consensus belief, before the present invention, is that the diaminoacridines have little or no value in limiting tumor growth and no therapeutic value as tumoricidal agents or in reducing the size of existing tumors. This erroneous belief has prevailed most likely because the toxicities of the diaminoacridines being used such as acriflavine were greater by 5 to 10 times than proflavine, the diaminoacridine used in the inventor's studies and which the present invention demonstrates to be a therapeutically effective tumoricidal agent. The pharmacological action of all diaminoacridines is similar differing primarily in terms of toxicity.

Prior art also teaches that one of the difficulties in the treatment of AIDS is that

macrophages act as a reservoir for the virus in the body. People with AIDS typically have tens of billions of infected macrophage cells. Existing treatments such as AZT prevent the virus from replicating in T-cells, but they do not affect the macrophages. Prevailing theory teaches that a substance which would destroy these infected, transformed macrophages would be very effective in the treatment of AIDS, particularly if that substance did not harm the normal macrophages in therapeutic doses.

It is also known that an anti-tumor chemotherapy, such as amethopterin, can be effective against other diseases associated with the growth of abnormal cells, such as arthritis or psoriasis, indicating that a substance which selectively kills abnormal cells will possibly be effective as a therapy for arthritis' and psoriasis.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a product and method for killing undesirable abnormal cells.

It is a further object of the present invention to provide a product and method which will not destroy normal cells.

Another object of the present invention is to provide a product and method for the treatment of cancer.

A further object of the present invention is to provide a product and method for the treatment of AIDS.

It is still another object of the present invention to provide a product and method for the treatment of rheumatoid arthritis.

Another object of the present invention is to provide a product and method for the treatment of psoriasis.

In accordance with the foregoing objectives, the present invention provides a method of treating diseases, such as cancer, AIDS, rheumatoid arthritis and psoriasis, by destroying abnormal cells by means of the administration of effective amounts of diaminoacridines. The diaminoacridines kill abnormal cells in tissue culture at concentrations as low as .85 mg. per kg. in the case of proflavine. The present invention includes the discovery that diaminoacridines such as proflavine sulfate and proflavine hemisulfate are not harmful to normal tissues at concentrations which will lyse all abnormal cells which were tested. Furthermore, the present invention includes the unexpected result that tumor cells and other abnormal cells are killed in the body by diaminoacridines such as proflavine hemisulfate within a few days with no apparent toxicity to the normal tissues of the body.

From previous uses of proflavine, it is known that it can be safely administered intravenously at a daily dosage of 3 mg. per kg. of body weight. It is known from studies of autopsied humans and sacrificed animals that proflavine, intravenously administered, reaches and stains all tissues except for nervous tissue. Proflavine lyses cells in tissue culture at concentrations below that at which it will stain the tissue. Therefore if proflavine can be kept at a concentration of at least .85 mg./kg. in the medium surrounding the abnormal cells within the body for a period from 6 to 48 hours, it should cause the lysis of all abnormal cells, without harming normal cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes the discovery that diaminoacridines in general, and proflavine hemisulfate in its preferred embodiment, are agents which are selectively toxic to abnormal cells without being harmful to normal tissues in effective doses. This is directly contrary to prior art teachings that diaminoacridines do not reduce tumor size or otherwise selectively harm abnormal tissues.

Tissue culture studies conducted in the development of the present invention led to the conclusion that proflavine sulfate and proflavine hemisulfate are effective in very low concentrations in killing cancer cells and other types of abnormal cells.

Ex. 1: Human fibrosarcoma tissue cells were cultured in Eagle's medium plus proflavine hemisulfate at dilutions of 1:1,125,000; 1:600,000; 1:120,000. At all dilutions, cells were lysed within 48 hours.

Ex. 2: Freshly explained chicken fibrosarcoma cells were cultured in Eagle's medium with proflavine sulfate at dilutions of 1:1,125,000; 1:600,000; 1:120,000. At all dilutions, the cells were all lysed within 9 hours.

Ex. 3: Freshly explanted tumor cells from the lungs, intestines and kidneys of chickens were cultured in Eagle's medium at dilutions of 1:1,125,000; 1:600,000; 1:120,000. At all dilutions, all cells of all types were

lysed within 36 hours.

Ex. 4: Rat uterus and muscle cells were cultured in Eagle's medium plus proflavine in dilutions of 1:1,125,000; 1:600,000; 1:125,000. Cells were stained but not damaged and retained full motility.

The results of Example 4 are consistent with toxicity studies of leucocytes, erythrocytes and other cell types done by other investigators. These experiments indicate that proflavine has a greatly selective toxic effect upon abnormal or malignant cells as compared with normal cells. That proflavine is a relatively non-toxic substance is also proved by the fact that it is currently used as a topical antiseptic especially for the treatment of deep wounds. Also, in the 1920's, proflavine was injected intravenously in England to thousands of patients as an anti-gonorrheal agent.

Ex. 5: Four terminal cancer patients with untreatable tumors were given 85% proflavine hemisulfate with organic salts in 1:1,000 dilution in normal saline intravenously. Dosage level was 100 mg. per 70 kg. of body weight administered in drip daily for four consecutive days.

Patient J.S.C. had a breast tumor with a measured metastasized tumor in the liver. After four days, mammography showed no evidence of breast tumor. A

liver scan showed a reduced liver tumor with the remainder of the tumor appearing diffuse. There was also some regeneration of the liver.

Patient H.C. suffered from multiple myeloma. He was disabled and in pain. After four weeks H.C. reported he had returned to work, after being off work for five years because of pain, and was free from pain. He is resisting a bone scan because of the pain involved with the procedure.

Patient M.C. suffered from breast tumors, which were palpable and measurable. After five weeks, there was a measurable reduction in the size of each tumor.

Patient A.W. suffered from a large squamous cell carcinoma which was in the neck, jaw and mouth and distorting the entire face. On the fourth day after the beginning of treatments, the tumor had been reduced over 50% in size.

The only negative effect of the proflavine was that the patients became somewhat sensitive to the sun. This is consistent with previous reports concerning the systemic use of proflavine. Also, the proflavine apparently exerts a somewhat anaesthetic effect at the point of administration. Normal saline will cause a burning sensation if there is infiltration into the surrounding tissue, alerting

the patient. The proflavine, when added to the normal saline, does not cause this burning sensation. In one case there was substantial infiltration before it was noticed, and an ulceration developed which took five weeks to heal. After this incident, the patients were first given normal saline. After it was certain that no infiltration was taking place, the proflavine solution was substituted for the saline.

Until the present invention it was believed that the intravenous injection of proflavine and other diaminoacridines was never justified and that proflavine and other diaminoacridines had no therapeutic effect upon existing tumors. The present invention comprises the unexpected result that proflavine is a highly selective toxic agent in its action against cancer tissue, though relatively harmless to normal cells. Furthermore, this invention comprises the surprising result that at therapeutic dosage levels, the action upon the cancer cells begins within four days of the first administration.

Until the present invention it was believed that the only substance which selectively killed or transformed infected macrophages in tissue culture was GLQZ33, a drug of unknown toxicity to humans. Infected macrophages are important reservoirs of the AIDS virus in humans.

Ex. 6: Transformed hen macrophages were grown in Tyrode's and blood serum plus proflavine in dilutions of 1:1,000,000; 1:500,000 and 1:125,000. All cells in these cultures were lysed after 20 hours. Tissues were not stained at

these concentrations.

Many studies have shown that proflavine has no effect either on motility or phagocytosis of stained normal macrophages in the mammalian body. Therefore it is believed that proflavine and other pharmacologically similar diaminoacridines are selectively toxic to transformed macrophages. Since these infected macrophages are believed to be the principal repository of the AIDS virus, a drug which can selectively kill these macrophages should be effective against AIDS.

Dosage levels can be similar to those used for cancer patients and may range from 1 mg. per kg. of body weight daily for four days to single doses of as much as 65 mg. per kg. of body weight.

Since abnormal cell growth is believed to be the cause of disease such as arthritis and psoriasis, and drugs such as methotrexate which are effective cytotoxic agents are effective against arthritis and psoriasis, it is believed that proflavine, which is selectively toxic to abnormal cells, will also be of benefit in stopping the abnormal cell growth found in arthritis and psoriasis. Since the selective toxicity of proflavine is much greater for the abnormal cell than is the selective toxicity of methotrexate, it is believed that proflavine will be an even more efficacious treatment for these diseases.

There are many other diaminoacridines in addition to proflavine which are known to have similar pharmacological action, such as acriflavine and diflavine. It is believed that the present invention can be utilized with any of the diaminoacridines though the preferred method is believed to utilize proflavine because of considerations of cost, availability and lack of toxicity to normal cells.

I CLAIM AS MY INVENTION:

1. A method of treating disease by destroying undesirable abnormal cells with a selectively toxic agent comprising administering an effective amount of diaminoacridine compound.

2. The method claimed in claim 1 wherein the diaminoacridine compound is chosen from the class consisting of proflavine; diflavine; 1,6 diaminoacridine; 3,9 diaminoacridine; 1,9 diaminoacridine; 2,7 diaminoacridine; 2,9 diaminoacridine; 3,5 diaminoacridine; 3,7 diaminoacridine; 4,5 diaminoacridine; and 4,9 diaminoacridine.

3. The method claimed in claim 1 wherein the diaminoacridine compound is administered in a dosage from 1 mg./kg. of body weight to 70 mg./kg. of body weight.

4. The method claimed in claim 1 wherein the diaminoacridine compound is administered intravenously.

5. The method claimed in claim 1 wherein the diaminoacridine compound is administered orally.

6. The method claimed in claim 1 wherein the diaminoacridine compound is administered parenterally.

7. A method for treating cancer comprising:
administering an effective amount of a diaminoacridine compound.

8. The method claimed in claim 7 wherein the diaminoacridine compound is proflavine.

9. The method claimed in claim 8 wherein the effective amount of proflavine is between 1 and 70 milligrams per kilogram of body weight.

10. A pharmaceutical preparation for use in the treatment of cancer, AIDS, rheumatoid arthritis and psoriasis comprising a diaminoacridine compound in a 1:1,000 dilution of normal saline solution in which the effectiveness of the diaminoacridine compound is increased because the toxicity of the diaminoacridine is controlled.

11. The pharmaceutical preparation claimed in claim 10 wherein the diaminoacridine compound is proflavine.

12. A method for treating AIDS, rheumatoid arthritis and psoriasis comprising:

administering an effective amount of proflavine.