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(54) Titre : UTILISATION DE PARVOVIRUS POUR AMELIORER L'ETAT GENERAL DE PATIENTS SOUFFRANT DE TUMEURS OU DE PATIENTS SOUFFRANT DE MALADIES CHRONIQUES OU DU TYPE CACHEXIQUE

(54) Title: USE OF PARVOVIRUSES FOR IMPROVING THE GENERAL CONDITION OF TUMOUR PATIENTS OR PATIENTS HAVING CHRONIC OR CONSUMPTIVE DISEASES

(57) Abrégé/Abstract:
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Abstract of the Disclosure

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Use of Parvoviruses for Improving the General Condition of Tumor Patients or Patients Having Chronic or Consumptive Diseases

The invention relates to the use of parvoviruses for improving the general condition of tumor patients or patients having chronic or consumptive diseases.

In addition to their diseases, tumor patients and patients having chronic or consumptive diseases often suffer from a poor general condition as well. This poor general condition manifests itself *inter alia* in abnormal blood values, fatigue, lack of energy and lack of appetite. This poor general condition is often worsened by a necessary therapy carried out with genotoxic agents. This applies in particular to patients suffering from malignant diseases. Along with a surgical removal, the therapy of malignant diseases is presently based predominantly on the application of genotoxic agents which as chemotherapeutic or radiotherapeutic agents attack and destroy tumor cells. As a rule, the death of the tumor cells is caused by triggering programmed cell death (apoptosis). However, the use of the genotoxic agents is limited by the occurrence of side-effects such as nausea, hyperemesis, depression of blood formation, gastrointestinal troubles, bleedings, *etc.* The genotoxic agents therefore cannot be used in a dose higher than permitted by the maximum tolerable side-effects. In many cases, the side-effects are unpleasant, or even life-threatening, for the patient so that the still tolerable doses of the genotoxic substance do not suffice to eliminate all of the tumor cells. The same applies to chronically inflammatory diseases, every form of autoimmune diseases or other indications in which genotoxic therapies, e.g. in the form of chemotherapeutic, radiotherapeutic or immunotherapeutic agents, are used. It is thus particularly desirable to provide possibilities reducing the side-effects occurring as a result of the

therapy so as to increase the effectiveness of the genotoxic agents. Such approaches should also improve the general clinical condition of tumor patients or patients suffering from chronic or consumptive diseases.

It is therefore the object of the present invention to markedly improve the general condition of tumor patients or patients suffering from chronic or consumptive diseases. The problem raised by side-effects induced by genotoxic therapy shall also be reduced. As a result, the genotoxic agents shall be usable in greater amount and also be tolerated better by the patient in higher doses. This adds to the efficiency of the therapy and improves the survival rate of patients.

This problem is solved by the subject matters defined in the claims.

According to the invention parvoviruses, in particular adeno-associated viruses, are used to reduce the side-effects which otherwise occur in patients who have to undergo genotoxic treatment. The general condition of the diseased individuals is also markedly improved. This applies in particular to tumor patients because the formation of tumor cachexia and bone-marrow depression caused by a neoplastic disease are markedly reduced.

The present invention is based on the observation that based on a genotoxic therapy the administration of parvoviruses markedly reduces the otherwise common side-effects. This is accompanied by an increased effect (in particular anti-neoplastic effect) of the therapy, since higher doses of genotoxic agent are then tolerated and the duration of the therapy can be extended. The effectiveness of a tumor treatment can be increased by a combined application of genotoxic therapy and administration of parvoviruses and the side-effects of the genotoxic treatment can simultaneously be reduced drastically. This effect is not reduced by the repeated application of the common administration of genotoxic agent and parvovivirus infection either. The effectiveness and

above all the compatibility of a chemotherapy or radiotherapy can be increased considerably by this approach.

According to the inventors' findings a parvovirus infection in combination with a genotoxic therapy, e.g. chemotherapeutic or radiotherapeutic measures, increases the efficiency of a conventional therapy and reduces side-effects occurring, so that another therapy is more promising than formerly. According to the invention any nonpathogenic parvoviruses can be used. Adeno-associated viruses (AAV) are particularly promising and thus preferred. It is also possible to use fragments of parvoviruses, in particular of AAV, as long as they have the desired effect. It is particularly preferred to use the AAV-2 virus or fragments thereof.

The AAV virus is a human parvovirus requiring co-infection with a helper virus for a productive infection to take place. AAV infects humans in early childhood and is considered nonpathogenic, since no human disease can be associated with the AAV infection (Adv. in Vir. Res. 1987, 32, 43-306). The AAV infection does not result in the formation of a neutralizing immunoresponse either, so that re-infections may occur at any time. Seroepidemological data show that tumoral diseases in patients having antibodies against AAV occur less often than in patients having no antibodies against AAV.

Animal experiments conducted with immunocompetent Lewis rats which were given pancreatic cancer cells by subcutaneous implantation showed that an AAV infection which was made at the same time as a chemotherapeutic therapy resulted in a tumor progression reduced as compared to the group of rats which had only been treated by means of chemotherapy. Furthermore, the rats which during the chemotherapy had simultaneously been given AAV-2 by means of infection showed a loss of weight markedly reduced to that of animals which had been treated by means of chemotherapy alone. It was also possible to markedly improve the vigilance of the animals by a combined administration of

AAV-2 and chemotherapy. The hematological parameters were also impaired in the AAV-2-infected animals by the chemotherapy to an extent markedly less than that of the animals which had been treated by means of chemotherapy alone.

The parvoviruses, in particular AAV viruses, can be used according to the invention without an additional treatment or be used before, at the same time as or after a genotoxic therapy. However, they are preferably used before the first genotoxic treatment and this use is repeated with each therapy cycle in which the genotoxic agent is administered. In this way it is possible to achieve at least partial remission in connection with tumors which were not treatable by the otherwise common usable doses of genotoxic agent. At the same time, the parovivirus infection considerably improves the entire physiological situation including the blood count and the maintenance of body weight.

The genotoxic therapy may preferably be any radiotherapy or chemotherapy which is adapted to the cancer to be treated. Genotoxic agents based on this therapy are the chemotherapeutic agents (see below) well known to a person skilled in the art and different irradiation sources, e.g. alpha, beta, gamma rays, neutron, proton or other types of rays. Such therapies have been known for years and along with the surgical removal of the tumor they are the established method of curing cancerous diseases or extending a patient's life expectancy for some time. Therefore, the person skilled in the art is well familiar with the steps of radiotherapy or chemotherapy. Along with the classical therapy, chemotherapy or radiotherapy, the invention also includes the combination of parvovirus infections with any other form of antineoplastic therapy. In particular the combination with gene-therapeutic approaches should be mentioned here. According to the invention the tumor therapy in any form of gene therapy or immunotherapy is supported by recombinant viruses as well as all the non-viral vectors and applications by the simultaneous administration of

parvoviruses. This includes in particular the transfer of what is called tumor suppressor genes or what is called suicide genes, the use of antibodies, cytokines and other medicaments of any kind or directed against tumor cells. This also comprises the therapy with all immunomodulatory approaches including all of the vaccination methods serving for building up an immunoresponse to the disease.

According to the invention the parvoviruses, in particular AAV viruses, can be used for any cancer types, maximum success being expectable with all of the gastrointestinal tumors and tumors of the respiratory tract and upper digestive system as well as the lower urinary tract, nervous system, genitals and all mesenchymal tissues and the hemopoietic system. Colon, pulmonary, pancreatic and brain tumors (in particular glioblastoma) are to be mentioned preferably. However, the invention also comprises all of the other indications in which chemotherapeutic or radiotherapeutic agents are used. This applies in particular to chronically inflammatory diseases (e.g. rheumatoid arthritis and further diseases of the rheumatic form), every form of autoimmune diseases (e.g. Crohn's disease, ulcerative colitis, *etc.*) or other indications in which chemotherapeutic, radiotherapeutic or immunotherapeutic agents are used. This also comprises all of the chronic consumptive diseases (e.g. tumoral diseases of any kind, HIV infections, *etc.*) in which the patients' general conditions can be improved by administering parvoviruses.

It also turned out that the sole administration of parvoviruses, in particularly AAV, without chemotherapy can markedly improve the condition of animals suffering from chronically consumptive diseases, such as a growing tumor. Therefore, the invention also relates to any form of administration of parvoviruses in the case of chronic, degenerative and/or consumptive states aiming at improving the general condition.

The use according to the invention is intravenous, subcutaneous, intra-arterial, intraperitoneal, intramuscular, intratumoral, peroral (also *per inhalationem*) or cutaneous. The parvovirus can also be realized directly at the site of disease by special drug application systems under pharmacological aspects. In this connection, the virus is formulated in a suitable preparation adapted to the pathway of administration. For example, for an intravenous (also as an infusion) and intratumoral administration it is preferred to provide the virus in a physiological common salt solution, Ringer's solution or PBS solution (phosphate-buffered salt solution), for a cutaneous administration it is preferred to provide it in the form of an ointment, suspension or gel, and for oral administration it is preferably provided in the form of tablets or dragées and for inhalation purposes as an aerosol spray.

Depending on the patient's weight the virus dose used is 10^9 - 10^{10} viral particles/kg body weight. However, the dose is determined by a physician and depends on the patient's weight, age and sex as well as the severity of the disease, kind of administration and duration of administration. A composition according to the invention can also contain conventional auxiliary agents. The auxiliary agents used may be the common ones, such as carriers, binders, blasting agents, lubricants, solvents, solutizers, release accelerators, release retarders, emulsifiers, stabilizers, colorants or taste corrigents.

According to the invention a pharmaceutical composition is also provided which along with a genotoxic agent, in particular a chemotherapeutic agent (cytostatic agent), contains parvoviruses, in particular adeno-associated viruses, e.g. AAV-2. All of the chemotherapeutic agents (cytostatic agents) common thus far in the tumor therapy or other therapies can be used as such or in combination as chemotherapeutic agents, e.g. cis-platinum and its derivatives, etopside, methothrexate, doxorubicin, cyclophospamide, ifosphamide, trofosfamide, busulfane,

cytarabine, fluorouracil, mercaptopurine, vinblastinesulfate, vincristinesulfate, bleomycinsulfate, taxol or mitomycin.

The invention is explained in more detail by means of the below example.

Example 1

In animal experiments carried out with immunocompetent Lewis rats, syngeneic DSL6A pancreatic cancer cells having a tumor cell number of $1x10^6$ were given to animals by subcutanoues inoculation into the groins thereof. These cells grew in all of the animals treated in this way to give tumors. After 4 or 6 weeks, the tumors had reached an average size of 30 mm^2 and 60 mm^2, respectively. Two different doses were used for 5-fluorouracil (5-FU), (5 mg/kg/body weight (minimum dose) or 50 mg/kg/body weight (maximum dose) weekly per animal). Animals having an average tumor size of 30 mm^2 four weeks after the tumor inoculation were treated with the minimum dose for 5-FU (5 mg/kg/body weight). In contrast thereto, animals having an average tumor size of 60 mm^2 six weeks after the tumor inoculation were treated with the maximum dose for 5-FU (50 mg/kg/body weight). In order to study the effect of a simultaneous AAV-2 infection, AAV-2 with $1x10^8$ viral particles was given intratumorally to each experimental animal once a week. Tumor-bearing animals without treatment served as a control group. In order to be able to control the effect of a sole AAV infection on tumor growth and the physiological changes in the experimental animals, tumor-bearing Lewis rats having $1x10^8$ AAV-2 viral particles were infected intratumorally (Table 1)

Numer of animals	Description
12	Control, without therapy
9	5-FU, dose 5 mg/kg/body weight (once per week, i.p.)
13	5-FU, dose 5 mg/kg/body weight (once per week, i.p.) + AAV-2 ($1x10^8$ viral particles weekly, i.t.)

12	5-FU, dose 50 mg/kg/body weight (once per week, i.p.)
12	5-FU, dose 50 mg/kg/body weight (one per week, i.p.) + AAV-2 ($1x10^8$ viral particles weekly, i.t.)
12	AAV-2 ($1x10^8$ viral particles weekly, i.t.)

The tumor size (length x width), occurrence of metastases and weight were determined for all of the animals once a week. According to the LASA (Laboratory Animal Science Association) working party recommendations a SCORE was developed and evaluated for the experimental animals once a week (Report of the laboratory animal science association working party, in Laboratory animals, 1990, (24), 97-130).

	3 scores	**2 scores**	**1 score**
Vigilance/behavior	Normal	Inactive or restless	Sleepy, unconscious
Posture/motoricity	Normal	Partially limited	Paralyzed, immobile
Coat of hair	Normal	Dull, bristled	Loss of hair
Nutritional condition	Normal	Reduced	Skinny, dehydrated
Pain	Little	Pressure-sensitive	Relieving posture, audible indications of pain

After 5-6 weeks of treatment, 1 ml blood was withdrawn from the caudal vein of 6 animals each from the different experimental groups and the hematological parameters, leukocyte number (total), hemoglobin, hematocrit, thrombocyte number and the differential blood count (neutrophilia, lymphocytes, monocytes) were studied.

The treatment was stopped when the weight reduced by > 20 %. The terminal point of the study was an expansive tumor size of > 400 mm^2 or the occurrence of more than 3 metastases.

RESULTS

In the course of this experiment, it turned out that the animals bearing the tumor and not treated (control group) showed rapid tumor growth. As a result, there was a loss of weight, leukopenia in the blood count and, as compared to healthy animals without tumor, a decrease in the polymorphonuclear granulocytes and monocytes, enhanced in terms of percentage, shown by the differential blood count. The animals which were given AAV-2 by way of infection showed age-adequate increase in weight in spite of progressive tumor growth and did not suffer from leukpenia. All in all, these animals were much more vital and had a significantly better LASA score for evaluating their general condition.

Table: Comparison between tumor-bearing animals without therapy and tumor-bearing animals with AAV infection after a maximum of 10 weeks after the tumor inoculation (average indication)

	Weight (g)	Leukocytes (per µl)	LASA score	Survival rate
Tumor-bearing animals without therapy	321	3067	3	0/12
Tumor-bearing animals with AAV infection	372	8700	13	8/12

When the average tumor size was 30 mm^2 four weeks after the tumor inoculation, the sole chemotherapy using 5-FU in a dosage of 5 mg/kg/body weight showed no inhibitory effect on tumor growth. In contrast thereto, the combinatory treatment with an equal dosage of 5-FU (5 mg/kg/body weight) and a simultaneous AAV infection was able to stop tumor growth for 2-3 weeks. Thereafter, tumor progression was reduced as compared to the animals treated exclusively with 5-FU (5 mg/kg/body weight). Thus, of 13 animals all were alive after 6 weeks of treatment, whereas 5 of 9 animals treated by means of chemotherapy

alone had died. Moreover, the animals additionally infected with AAV had a better LASA score and a larger number of leukocytes than the animals treated exclusively by means of chemotherapy.

Table: Comparison between 5-FU monotherapy (5 mg/kg/body weight, minimum dose) and combinatory treatment of 5-FU (5 mg/kg/body weight) with simultaneous AAV infection after 6 weeks of treatment (average indication).

	Weight (g)	Leukocytes (per µl)	LASA score	Survival rate
5-FU (5 mg/kg/-body weight)	355	6,843	9	4/9
5-FU (5 mg/kg/-body weight + AAV-2	354	9,500	15	13/13

* Commencement of treatment four weeks after the tumor inoculation (TU size ~ 30 mm^2)

Six weeks after the tumor inoculation, the tumors had reached an average size of 60 mm^2. The 5-FU chemotherapy with the maximum dose of 50 mg/kg/body weight was either given to these animals as a monotherapy or in combination with the AAV infection. Here, a short-term stand-still of tumor growth was achieved for the animals treated exclusively with 5-FU. In the further course of therapy, considerable side-effects of chemotherapy manifested themselves. For example, due to the poor general condition of the animals and an average reduction in weight of over 20 % the therapy had to be interrupted after the second week of treatment. Animals having an additional AAV infection did not have these side-effects and initially showed tumor decrease. Thereafter, the tumor growth stopped for about 4 weeks and the survival period of the rats was prolonged significantly as compared to that of the controlled animals or animals treated exclusively with 5-FU. After 6 weeks of treatment, 9 of 12 rats treated exclusively with a maximum dose given on the basis of

chemotherapy had died. In contrast thereto, only 3 of a total of 12 rats had died in the group of animals infected additionally with AAV.
When the parameters showing the course were determined 5 to 6 weeks after the commencement of the therapy it turned out that animals treated by means of chemotherapy without simultaneous AAV-2 infection suffered from distinct leukopenia, a reduced LASA score and a markedly reduced weight. Animals which had been given both chemotherapy and AAV infection did not show chemotherapy-induced or tumor-induced leukopenia. These animals were much more vital (LASA score) and did not lose weight.

Table: Comparison between 5-FU monotherapy (50 mg/kg/body weight, maximum dose) and combinatory treatment of 5-FU (50 mg/kg/body weight) with simultaneous AAV infection after 6 weeks of treatment (average indication).

	Weight (g)	Leukocytes (per µl)	LASA score	Survival rate
5-FU (50 mg/kg/body weight)	306	640	6	3/12
5-FU (50 mg/kg/body weight) + AAV-2	353	8,325	14	9/12

* Commencement of treatment six weeks after the tumor inoculation (TU size ~ 60 mm^2)

The significantly better general condition of the animals additionally infected with AAV and the thus reduced side-effects of maximum-dose chemotherapy (5-FU, 50 mg/kg/body weight) manifested itself in all of the investigated hematological parameters. For example, the animals treated exclusively with 5-FU (50 mg/kg/body weight) showed significantly lowered values for hemoglobin, the thrombocyte number and the values for neutrophilia, lymphocytes and granulocytes, determined in the differential blood count.

Table: Comparison between the hematological parameters of 5-FU monotherapy (50 mg/kg/body weight, maximum dose) and those of 5-FU (50 mg/kg/body weight) with simultaneous AAV infection after 6 weeks of treatment (average indication).

	Hemo-globin (g/dl)	Thrombo-cytes (per µl)	Neutro-philia (per µl)	Lympho-cytes (per µl)	Monocytes (per µl)
5-FU (50 mg/kg/body weight)	6.5	76,000	18	606	6
5-FU (50 mg/kg/body weight) + AAV-2	11.6	540,000	353	6,503	1,002
Standard values Rat	13.5	621,000	820	9,100	220

* Commencement of treatment six weeks after the tumor inoculation (TU size ~ 60 mm^2)

These studies show that the accompanying infection of tumor-bearing rats with adeno-associated viruses (e.g. AAV-2) considerably improves the blood count as well as the entire physiological condition and reduces the loss of weight of the animals.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of non-pathogenic parvoviruses for the production of a medicament for reducing the side-effects of therapies with genotoxic agents in patients suffering from a tumoral disease, the reduction of the side-effects occurring while the dose of the genotoxic agents is maintained or raised and the side-effects relate to hematological parameters.

2. Use of non-pathogenic parvoviruses for reducing the side-effects of therapies with genotoxic agents in patients suffering from a tumoral disease, the reduction of the side-effects occurring while the dose of the genotoxic agents is maintained or raised and the side-effects relate to hematological parameters.

3. Use according to claim 1 or 2, wherein the parvoviruses used are adeno-associated viruses.

4. Use according to claim 3, wherein the adeno-associated viruses are AAV-2.

5. Use according to any one of claims 1 to 4, wherein the parvoviruses are to be used in a dose of 10^9 - 10^{10} particles/kg body weight.

6. Use according to any one of claims 1 to 5, wherein the parvoviruses are to be used intravenously, subcutaneously, orally, intraperitoneally, intraarterially or intratumorally.

7. Use according to any one of claims 1 to 6, wherein the parvoviruses are to be used before, or simultaneously with, the therapy with genotoxic agents.

8. Use according to any one of claims 1 to 7, wherein the genotoxic agents are to be used based on a chemotherapy, radiotherapy, a gene therapy or an immunotherapy.