The Curative and Palliative Potential of the Monoclonal Antibody MOv18 Labelled with ^{211}At in Nude Mice with Intraperitoneally growing Ovarian Cancer Xenografts

A Long-Term Study

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The purpose of the present study was to investigate the therapeutic efficacy of ^{211}At-labelled specific monoclonal antibody MOv18 in nude mice with intraperitoneal growth of the human ovarian cancer cell line OVCAR3. In the first part of the study the antibody was injected intraperitoneally when the cancer growth was microscopic. The injected activity was 485–555 kBq. The median survival for treated mice was 213 days compared to 138 days for untreated mice ($p < 0.014$, log-rank test). No obvious toxicity was seen. Thirty-three percent of the mice were apparently free of cancer after 7 months and were probably cured. In the second part of the study mice with macroscopic cancer and signs of ascites were injected intraperitoneally with the same ^{211}At-labelled antibody (377–389 kBq). This treatment possibly delayed the production of ascites. Hopefully radioimmunotherapy with regionally administered ^{211}At-labelled antibody will be of value in women with ovarian cancer as well.

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At the time of diagnosis human ovarian cancer has spread beyond the ovaries in about 75% of afflicted patients. In most cases the dissemination is restricted to the peritoneal surface (FIGO stage IIB and III) and is well suited for local treatment. The clinical response rate with intravenous combination chemotherapy is high but even when a pathologically complete remission is obtained up to 50% of the patients will have a tumour relapse (1) due to undetected microscopic cancer. There is thus a great need for consolidating treatment after the systemic chemotherapy. Intraperitoneal chemotherapy (2) and whole abdomen irradiation (3) have been used with some success but with considerable toxicity. Intraperitoneal administration of a specific monoclonal antibody labelled with a radionuclide is an attractive treatment as the antibody binds specifically to the cancer cells and brings the isotope into close proximity. However, as the capacity of the antibody to diffuse into a tumour nodule is very restricted, this treatment is probably only suitable for micrometastases. There are a few promising reports on clinical radioimmunotherapy of minimal residual ovarian cancer with specific antibodies labelled with the β-emitters 90Y or 131I (4, 5). However, α-emitters with a higher RBE and a much shorter range in tissue are better suited for the treatment of micrometastases (6). In a previous short-term study (7) we found that when nude mice with intraperitoneally growing human ovarian cancer were treated with intraperitoneal injection of the specific antibody MOv18 labelled with ^{211}At, a large proportion of the animals became tumour-free. However, the long-term survival, which both experimentally and clinically is more important than the response rate, was not studied.

Moreover, in the later stages of ovarian cancer resistent to chemotherapy, ascites can be a difficult clinical problem, causing discomfort and distress to the patients and necessitating repeated paracentesis. Intraperitoneally administered chemotherapy can have a palliative effect (8) but there are also reports of decreased ascites production

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with minimal toxicity after intraperitoneal administration of ^{131}I-labelled antibody (9, 10).

The objective of the present study was to estimate the survival of female nude mice with microscopic intraperitoneally growing human ovarian cancer after intraperitoneal treatment with the antibody MOv18 labelled with ^{211}At. Furthermore, we have investigated the effect of ^{211}At-labelled antibody on the ascites production in mice with more advanced disease.

MATERIAL AND METHODS

Monoclonal antibodies

MOv18 is a murine monoclonal antibody first characterized by Miotti et al. It recognizes a membrane folate-binding glycoprotein of 38 kDa and reacts with about 90% of human ovarian carcinomas (11–13).

Conjugate ^{211}At-labelling of antibodies

The labelling of m-MeATE was performed by modification of the method earlier described (14). Briefly, ^{211}At, 20–30 MBq in chloroform, was added to a 1.3 ml reaction vial and the solvent was evaporated to dryness under a gentle stream of nitrogen. Astatine was resolved in 1.5 µl of 0.05 M NaOH, 15–20 nmol of m-MeATE and 35 nmol chloramine-T in dimethylformamide (DMF)/water/acetic acid (HAc), 24 : 75 : 1 by volume, was added to final volume of 12 µl. The reaction mixture was incubated for 15 min during gentle agitation at room temperature. The labelling reaction was stopped by adding 70 nmol of $Na_2S_2O_5$. To the crude labelling mixture 100 µg of MOv18, 3–5 mg/ml, in 0.2 M carbonate buffer pH 8.5 was added. The conjugation reactions were allowed to proceed for 30 min during gentle agitation at room temperature. The antibody fraction was finally isolated by passage over a Sephadex G-25 pD-10 column (Pharmacia, Sweden). Radiochemical purity of labelled antibody was determined by trichloric acid, TCA, precipitation and FPLC analysis.

Immunoreactivity

In a previous study, the results of the immunoreactivity test for labelled MOv18 varied considerably (15). Other authors have also reported low and variable figure probably due to a high spontaneous release of the antigen recognized by MOv18 (16, 17). Therefore, we did not attempt to determine the immunoreactivity in the survival study. Later we used fixed cells grown in a folate-free medium and the immunoreactive fraction of the labelled antibodies in the second part of the study was 0.74 according to Lindmo et al. (18).

Tumours and animals

Xenografts were established from the human ovarian cancer cell line NIH : OVCAR 3 (19) by injection of 1×10^7 cells intraperitoneally in 6–8 week old female Balb/c nu/nu mice. Two weeks later there was microscopic peritoneal tumour-growth but no ascites. Two months after the injection of cancer cells the disease was well established in untreated mice with macroscopic tumour nodules and ascites in at least 90% of the animals. However, the tumour growth rate varied considerably between individual animals. At the beginning, the ascites was very viscous and impossible to aspirate and measure. Later when the abdomen was distended the ascites was more liquid and bloody. Except when the cancer was very advanced, the mice seemed to be thriving.

Biodistribution

The biodistribution of labelled MOv18 in nude mice with subcutaneous tumours has been presented in a previous study (15). There was a selective uptake in tumour tissue with a tumour to blood ratio of 2.2 after 72 h. Likewise in mice with macroscopic intraperitoneal tumour-growth an uptake of around 5% ID/g in tumour tissue and a tumour to blood ratio of 1.3 was seen 1 h and 3 h after intraperitoneal injection of labelled antibody (unpublished data).

STUDY DESIGN

Effect of ^{211}At -labelled antibody on the survival

Thirty mice were inoculated intraperitoneally with 1×10^7 OVCAR-3 cells. After 14 days 15 animals were randomly selected and injected intraperitoneally with MOv18 labelled with ^{211}At (458–555 kBq) while 15 mice were untreated. The administered activity was measured with a dose calibrator (Capintech, USA). The antibody (15 µg) was given intraperitoneally in 1 ml normal saline. The mice were sacrificed, when the general condition deteriorated or the abdomen was distended. They were dissected and the amount of tumour and ascites was estimated. Seven months after the start of the study the remaining mice were sacrificed. If they were macroscopically tumour-free, multiple biopsies were taken from the peritoneum for histopathologic examination. The survival time was estimated with the Kaplan–Meier method. The log-rank test was used for comparision between the groups.

Effect of ^{211}At-labelled antibody on ascites production

Two groups composed of 20 animals were used. Seventy-six days after the intraperitoneal inoculation of cancer cells 10 mice were randomly selected, weighed and injected intraperitoneally with 13.8 µg ^{211}At-labelled monoclonal antibody (377–389 kBq). The mean weight was 25.3 g (range 23.0–28.2). The other 10 mice with mean weight 25.5 g (range 21.0–30.0) were used as controls. There were signs of ascites in eight animals in each group. The mice were inspected and the weight was followed weekly and the weight increase was compared between the two groups. If the abdomen was distended and the general condition deteriorated during the study period the mice were sacrificed.

RESULTS

Effect of ^{211}At -labelled antibody on survival

Survival among the two groups is shown in the Fig. 1. The untreated mice were sacrificed after 138 (median, range = 110–222) days. In 13 of them the abdomen was distended by ascites and extensive intraperitoneal tumour. The remaining had multiple small tumours but no ascites. Of the treated animals five (33%) were free of ascites and tumour when they were sacrificed 7 months after the inoculation of cancer cells. Microscopically no tumour was seen in the biopsies and they were considered cured. One mouse had a few intraperitoneal tumours with a size of up to 3 mm and two had large tumours in the upper left quadrant of the abdomen but no ascites at the same time-point. The remaining seven mice were sacrificed due to their cancer. All of them had intraperitoneal tumour masses, five had ascites and two had subcutaneous metastases with a maximal diameter of about 2 cm. The median survival time for all treated animals was 213 (123–223) days. The difference between the two groups was statistically significant ($p = 0.014$).

Effect of ^{211}At-labelled antibody on ascites production

The weight of the animals was followed for 5 weeks after the injection of ^{211}At-labelled antibody and the weight increase for each mouse was calculated. One mouse in each group was sacrificed before the end of the study period due to rapid increase of ascites, and for these individuals the weight increase up to their death was calculated.

In astatine-treated mice the weight was rather stable for the study period and increased only slightly except for one mouse (see above). The weight increase was 1.4 ± 1.0 g (mean $\pm$ SD, range 0.0–3.8 g). On the contrary, in untreated mice, the weight increase was greater and varied much more (4.1 ± 4.6 g; mean + SD, range –0.4–11.0 g), although the difference was not statistically significant. Increasing weight always corresponded to signs of an increasing amount of ascites on examination.

DISCUSSION

In the present study we have investigated the survival of nude mice with intraperitoneal growth of the human ovarian cancer line NIH : OVCAR 3 after treatment with the specific ^{211}At-labelled monoclonal antibody MOv18 administrated intraperitoneally. The treatment was given once 2 weeks after the inoculation of cells at which time the tumour was still microscopic. The median survival was 213 days compared to 138 days for untreated mice. Five (33%) of 15 mice were macro- and microscopic tumour-free and probably cured of their cancer.

In a previous short-term study we reported good therapeutic efficacy of the ^{211}At-labelled monoclonal antibody MOv18 under the same experimental conditions with 9 of 10 mice tumour-free about 6 weeks after treatment (7). When the unspecific antibody ^{211}At-C242 was used or the ^{211}At-MOv18 was injected intravenously the efficacy was less pronounced. As expected the long-term effect was comparatively worse and apparently it is difficult to eradicate ovarian cancer both experimentally in mice and clinically in women. There were no treatment-related deaths and the administered activity can probably be increased without undue toxicity. According to Larsen et al. (20) the maximum tolerated dose of intraperitoneally injected ^{211}At-labelled antibody may be between 50 and 100 kBq/g bodyweight, i.e. twice to four times the dose in the present study. Repeated injection of the labelled antibody could also have given a better result. However, this is problematic clinically with the unmodified antibody due to a human anti-mouse antibody (HAMA) response.

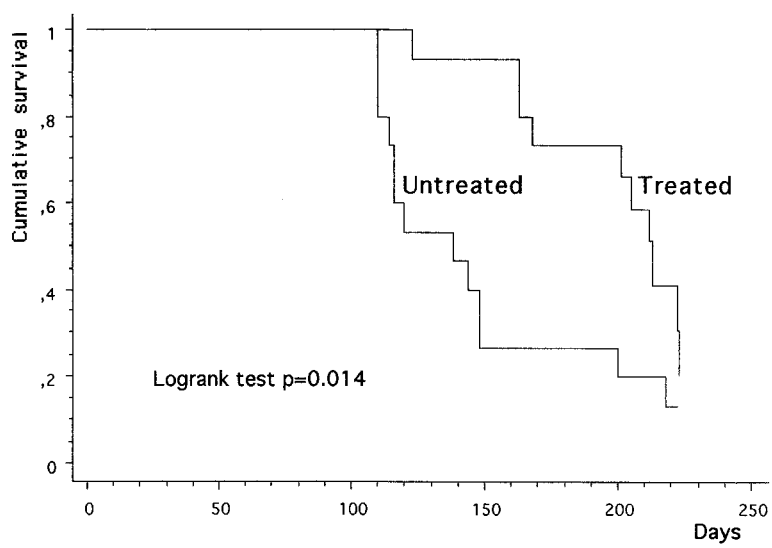

Fig. 1. Survival of treated compared to untreated mice. Mice sacrificed after 7 months were censored.

As far as we know there is no other report on the treatment of intraperitoneal ovarian cancer with a specific antibody labelled with ^{211}At in mice or in humans. However, Bloomer et al. (21) treated mice with intraperitoneally growing murine ovarian cancer with ^{211}At-tellurium colloid and compared it with radiocolloids of ^{32}P, ^{165}Dy and ^{90}Y. The radiocolloid was injected 24 h after the inoculation of cells. An injected activity of 925 kBq ^{211}At was curative. The β-emitters were not curative but prolonged the survival when large doses were given. Larsen et al. (20) have studied the therapeutic effect of ^{211}At-labelled monodisperse polymer particles in a murine intraperitoneal tumour model and compared it with labelled unspecific antibodies and free ^{211}At. The survival varied according to the injected activity and substance but was for most groups better than for untreated animals. No specific antibody was used in this experiment and the mice were injected 30 h after the inoculation of tumour cells. Utilizing another α-emitter, Horak et al. (22) injected SK-OV-3 cells intraperitoneally and 3 days later the anti-HER2/neu monoclonal antibody AE1 labelled with ^{212}Pb. The tumour-free survival was significantly prolonged compared to no treatment or treatment with unlabelled antibody.

In the previous three studies named above (20–22) the interval between the inoculation of cells and the treatment was short compared to the present study and many of the tumour cells would be expected to still be free-floating.

Clinically Hird et al. (4) have treated 52 patients with the ^{90}Y-labelled monoclonal antibody HMFG-1 after conventional surgery and chemotherapy. When the treatment was given to 21 patients without evidence of disease at the second-look laparotomy, the result was very promising with only two deaths after a median follow-up of 35 months.

Likewise Crippa et al. (5, 23) have treated patients with minimal residual ovarian cancer with encouraging results. They have used the MOv18 antibody labelled with ^{131}I.

Even if it is difficult to study, the ascites production was possibly delayed after intraperitoneal injection of ^{211}At-labelled monoclonal antibody into nude mice with advanced tumour. However, the individual variation – especially between untreated mice was great, the number of animals was small and there was no statistically significant difference between the groups. The same therapeutic effect has been seen clinically with ^{131}I-labelled antibody given to women with chemotherapy resistant ovarian cancer (9, 10, 24). The side effects in these studies were mild. Intraperitoneal chemotherapy with cis-platinum can control the ascites production but the toxicity is considerable with delayed nausea and vomiting (8, 25). Other forms of medical treatment that have been tried with some success in women include intraperitoneal radiocolloids (26), tumour necrosis factor alpha (27) and interferon (28).

In this report TCA precipitation together with FPLC analysis have been used in determining the radiochemical purity of the astatinated antibodies. It should be noted that TCA precipitation is not a valid method for measuring protein-associated ^{211}At activity since non-bound ^{211}At will co-precipitate with the protein as recently reported by Reist et al. (29). However, in this study there was no difference between the two analytical methods, due to the high protein-associated ^{211}At activity.

In summary, the long-term survival of nude mice with intraperitoneally growing human ovarian cancer was good with some mice apparently demonstrating cure. It seems feasible that this treatment could be given to women with minimal residual disease after conventional therapy given that there is no unacceptable toxicity and the efficacy is similar. Furthermore, the ^{211}At-labelled antibody can probably be valuable for the palliative treatment of ascites when the cancer is chemotherapy resistant.

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REFERENCES

1. Bolis G, Villa A, Guarnerio P, et al. Survival of women with advanced ovarian cancer and complete pathologic response at second-look laparotomy. Cancer 1996; 77: 128–31.
2. Markman M. Intraperitoneal therapy of ovarian cancer. Semin Oncol 1998; 25: 356–60.
3. Thomas GM. Is there a role for consolidation or salvage radiotherapy after chemotherapy in advanced epithelial ovarian cancer? Gyn Oncol 1993; 51: 97–103.
4. Hird V, Maraveyas A, Snook D, et al. Adjuvant therapy of ovarian cancer with radioactive monoclonal antibody. Br J Cancer 1993; 68: 403–6.
5. Crippa F, Bolis G, Seregni E, et al. Single-dose intraperitoneal radioimmunotherapy with the murine monoclonal antibody I-131 MOv18: clinical results in patients with minimal residual disease of ovarian cancer. Eur J Cancer 1995; 31A: 686–90.
6. Zalutsky MR, Bigner DD. Radioimmunotherapy with alpha-particle emitting radioimmunoconjugates. Acta Oncol 1996; 35: 373–9.
7. Andersson H, Lindegren S, Bäck T, Jacobsson L, Leser G, Horvath G. Radioimmunotherapy of nude mice with intraperitoneally growing ovarian cancer xenograft utilizing ^{211}At-labelled monoclonal anti-body MOv18. Anticancer Res 2000; 20: 459–62.
8. Markman M, Cleary S, Lucas WE, Howell SB. Intraperitoneal chemotherapy with high-dose cisplatin and cytosine arabinoside for refractory ovarian carcinoma and other malignancies principally involving the peritoneal cavity. J Clin Oncol 1985; 3: 925–31.
9. Buckman R, De Angelis C, Shaw P, et al. Intraperitoneal therapy of malignant ascites associated with carcinoma of ovary and breast using radioiodinated monoclonal antibody 2G3. Gynecol Oncol 1992; 47: 102–9.
10. Ward B, Mather S, Shepherd J, et al. The treatment of intraperitoneal malignant disease with monoclonal antibody guided ^{131}I radiotherapy. Br J Cancer 1988; 58: 658–62.

11. Boerman OC, van Niekerk CC, Makkink K, Hanselaar TGJM, Kenemans P, Poels LG. Comparative immunohistochemical study of four monoclonal antibodies directed against ovarian carcinoma-associated antigens. Int J Gyn Path 1991; 10: 15–25.
12. Miotti S, Canevari S, Me'nard S, et al. Characterization of human ovarian carcinoma-associated antigens defined by novel monoclonal antibodies with tumor-restricted specificity. Int J Cancer 1987; 39: 297–303.
13. Campbell IG, Jones TA, Foulkes WD, Trowsdale J. Folate-binding protein is a marker for ovarian cancer. Cancer Res 1991; 51: 5329–38.
14. Lindegren S, Skarnemark G, Jacobsson L, Karlsson B. Chloramine-T in high-specific-activity radioiodination of antibodies using N-succinimidyl-3-(trimethylstannyl)benzoate as an intermediate. Nucl Med Biol 1998; 25: 659–65.
15. Andersson H, Lindegren S, Bäck T, Jacobsson L, Leser G, Horvath G. Biokinetics of the monoclonal antibodies MOv 18, OV 185 and OV 197 labelled with ^{125}I according to the m-MeATE method or the Iodogen method in nude mice with ovarian cancer xenografts. Acta Oncol 1999; 38: 323–8.
16. Mantovani L, Me'nard S, Mezzanzanica D, Miotti S, Pupa SM, Colnaghi MI. Evaluation of the immunoreactive fraction of an anti-tumour monoclonal antibody. Br J Cancer 1990; 62 (Suppl X): 15–7.
17. Miotti S, Alberti S, Facheris P, et al. Membrane association and shedding of the GPI-anchored Ca-MOv18 antigen in human ovary carcinoma cells. Int J Cancer 1992; 51: 499–505.
18. Lindmo T, Boven E, Cuttitta F, Fedorko J, Bunn Jr PA. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. J Immunological Methods 1984; 72: 77–89.
19. Hamilton TC, Young RC, Louie KG, et al. Characterization of a xenograft model of human ovarian carcinoma which produces ascites and intraabdominal carcinomatosis in mice. Cancer Res 1984; 44: 5286–90.
20. Larsen RH, Hoff P, Vergote IB, et al. Alpha-particle radiotherapy with ^{211}At-labeled monodisperse polymer particles, ^{211}At-labeled IgG proteins, and free ^{211}At in a murine intraperitoneal tumor model. Gyn Oncol 1995; 57: 9–15.
21. Bloomer WD, McLaughlin WH, Lambrecht RM, et al. ^{211}At -radiocolloid therapy: further observations and comparison with radiocolloids of ^{32}P, ^{165}Dy, and ^{90}Y. Int J Radiat Oncol Biol Phys 1984; 10: 341–8.
22. Horak E, Hartmann F, Garmestani K, et al. Radioimmunotherapy targeting of HER2/neu oncoprotein on ovarian tumor using lead-212-DOTA-AE1. J Nucl Med 1997; 38: 1944–50.
23. Bombardieri E, Ferrari L, Spinelli A, Maffioli L, Seregni E, Buraggi G. Radioimmunotherapy of ovarian cancer with radiolabelled monoclonal antibodies: biological basis, present status and future perspectives. Anticancer Res 1997; 17: 1719–30.
24. Epenetos AA, Hooker G, Krausz T, Snook D, Bodmer WF, Taylor-Papadimitriou J. Antibody-guided irradiation of malignant ascites in ovarian cancer: a new therapeutic method possessing specifiicity against cancer cells. Obstet Gyn 1986; 68 (Suppl 3): 71S–74.
25. Lind SE, Cashavelly B, Fuller AF. Resolution of malignant ascites after intraperitoneal chemotherapy in women with carcinoma of the ovary. Surg Gyn Obstet 1988; 166: 519–22.
26. Card RY, Cole DR, Henscke UK. Summary of ten years of the use of radioactive colloids in intracavtary therapy. J Nucl Med 1960; 1: 195–8.
27. Räth U, Kaufmann M, Schmid H, et al. Effecy of intraperitoneal recombinat human tumour necrosis factor alpha on malignant ascites. Eur J Cancer 1991; 27: 121–5.
28. Bezwoda WR, Seymour L, Dansey R. Intraperitoneal recombinant interferon-alpha 2b for recurrrent malignant ascites due to ovarian cancer. Cancer 1989; 64: 1029–33.
29. Reist CJ, Foulon CF, Alston K, Bigner DD, Zalutsky MR. Astatine-211 labeling of internalizing anti-EGFRvIII monoclonal antibody using N-succinimidyl 5-[^{211}At]astato-3-pyridinecarboxylate. Nucl Med Biol 1999; 26: 405–11.