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Immunotherapy of colon cancer metastases in rats with the immunostimulant OM-89

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In a model of colon cancer in rats the bacterial extract OM-89 was found to have antitumor properties. We have shown that OM-89 induced the complete regression of numerous peritoneal tumor nodules measuring 1–5 mm in 46% of rats, and inhibited the growth of microscopic tumors in 42% of rats. OM-89 was effective when injected ip at the dose of 50 mg/kg, twice a week for 2 weeks. It was ineffective at the same dose when given orally every day for 14 or 21 days. The traces of endotoxins (0.0005%) were probably not involved in the antitumor effect of OM-89. No side-effects were observed.

Key words: Bacterial extract, colon cancer, experimental metastasis, immunotherapy.

Introduction

Up to 60% of patients with colon cancer may have microscopic and/or clinically evident metastases at the time of primary tumor diagnosis. Many treatments have been evaluated in advanced disease or as adjuvant to surgery, but none has prolonged the survival, except for a weak effect when levamisole and 5-fluorouracil were used together in patients with Duke'C colon cancer.[1] Colon cancer metastases are localized in the liver, lungs, ganglia and peritoneum, all of which are rich in macrophages. Macrophages are capable of killing cancer cells and they present to T lymphocytes partially degraded antigens from tumor cells whose debris they have phagocytosed. In addition, macrophages are capable of secreting a number of cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor (TNF) and growth factors (colony-stimulating factors) which regulate antitumor immunity and/or are toxic to cancer cells. These functions, which are specific to activated macrophages, may fail during tumor growth. OM-89 is a bacterial extract able to activate macrophages *in vitro*.[2] The aim of this study was to test *in vivo* the antitumor effect of OM-89 in an experimental model of colon cancer.

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Materials and methods

Rats, tumor cells and peritoneal carcinomatosis

BDIX rats, 3- to 5-month-old males or females, were purchased from the breeding colony of CNRS, Orléans, France. The tumor cell clone Pro b, syngeneic to BDIX rats, has been described previously.[3] It was obtained from a chemically induced colon adenocarcinoma and kept in culture. Cells were detached by EDTA and trypsin. The absence of contamination by bacteria, mycoplasma or fungi was checked before each *in vivo* injection by fluorescent staining of extranuclear DNA with bisbenzimide. All BDIX rats were injected ip with 10^6 viable Pro b cells. As reported previously,[4] under these conditions all the untreated control rats exhibited peritoneal carcinomatosis and haemorrhagic ascites and died of their tumors between the 8th and the 12th week.

Test compounds

LPSw from *Escherichia coli* (O 128:B12) was purchased from Difco Laboratories (Detroit, MI, USA). OM-89 was provided by OM Laboratories (Meyrin, Geneva, Switzerland). OM-89 is a

proteinaceous extract taken from *E. coli*. The experiments described hereafter were performed using several lots of OM-89 manufactured several months apart; all of them displayed similar activities. OM-89 was used either as a liquid containing 20 mg of active substance/ml, or as a lyophilized powder containing 10% of active substance and 90% of inert carriers. Both preparations were tested for endotoxin contamination by the *Limulus* amoebocyte lysate. They were found to contain less than 60 ppb of endotoxin (60 ng of LPS/ml of liquid concentrate) and less than 1.2 ppm of endotoxin (1.2 μg of LPS/g of lyophilized powder).

Immunotherapy

The treatment was started either on day 3 or on day 14 after tumor cell injection. At that time, all rats had either dividing cancer cells found histologically in the milky spots or macroscopic nodules found in omentum and mesentery. This was periodically checked on three rats injected with tumor cells on which autopsy was carried out at day 14.

The rats were treated either intraperitoneally or orally: rats received intraperitoneal injections of OM-89 dissolved in 0.9% NaCl, twice a week for 2 weeks (total of five injections), or they received OM-89 dissolved in water administered by gavage every day for 14 or 21 days. Experiments took 6 weeks and ended before the death of control rats. An autopsy was carried out on all rats at the end of each experiment.

Peritoneal carcinomatosis was scored on a scale from 0 to 4. Scoring was performed in a blind fashion. Rats were taken without reference to their ear tags and scored after inspection of their abdominal cavity. Rats of similar scores were placed in defined classes of peritoneal carcinomatosis. Class 0 was defined as no visible nodules, class 1 as few nodules less than 2 mm, class 2 as nodules less than 5 mm and too numerous to count, class 3 as peritoneal cavity invaded with nodules up to 1 cm, and class 4 as peritoneal cavity fully invaded with tumor masses, some of which measured several centimeters in diameter. After scoring, the ear tags were read.

The number of rats per treatment group was 10. The significance of the treatment effect on the peritoneal carcinomatosis and on the production of ascites was analyzed by a Kruskall–Wallis test or a variance analysis.

Results

Intraperitoneal treatment

Microscopic peritoneal carcinomatosis. At the doses of 10–100 mg of active substance/kg, OM-89 significantly prevented the growth of carcinomatosis and the production of ascites (Table 1). Cumulative data show that at autopsy 17 out of 40 treated rats had no tumors (class 0, 6 weeks after the tumor cell injection) whereas at the same time all the untreated control rats had tumors. In the 23 other rats OM-89 significantly slowed down the tumor growth and the ascites production. At the dose of 50 mg of active substance/kg, the antitumor effect of OM-89 was reproduced in two out of two experiments.

Table 1. Dose effect of OM-89 on the growth of microscopic peritoneal carcinomatoses induced by colon cancer cells in rats, data of a representative experiment

Treatment	Number of rats with carcinomatosis of the following classes					Effect of the treatment[a]	Volume of the ascites (ml/rat)		Effect of the treatment[b]
	0	1	2	3	4		Limit	Mean ± *SD*	
None	0	1	4	1	4		0–57	15 ± 20	
OM-89 10 mg/kg	2	2	4	1	1	$p < 0.01$	0–8	1 ± 2	$p < 0.001$
OM-89 50 mg/kg	4	3	1	1	1	$p < 0.01$	0–16	2 ± 1	$p < 0.001$
OM-89 100 mg/kg	3	6	0	0	0	$p < 0.01$	0–0	0 ± 0	$p < 0.001$

[a] Kruskal–Wallis test.
[b] Variance analysis.

Macroscopic peritoneal carcinomatosis. Dose–response experiments showed the best activity at a dose of 50 mg of active substance/kg. At this dose, in 14 out of 30 treated rats OM-89 induced the complete regression (class 0, 6 weeks after the tumor cell injection) of macroscopic carcinomatosis constituted of numerous nodules measuring 1–5 mm, whereas at the same time all the untreated rats had tumors. In the other 17 rats OM-89 significantly slowed down the tumor growth. Furthermore, OM-89 significantly inhibited the production of ascites. The antitumoral effect of OM-89 was reproducible in three out of three experiments (Table 2).

Comparison with antitumor activity of LPS from E. coli. The results in Table 3 show that endotoxin at a dose of 100 μg/kg induced a complete regression of macroscopic tumors in two out of ten rats, significantly slowed down the tumor growth in the other rats and inhibited the ascites production. At the doses of 1 and 10 μg/kg endotoxin was no more effective. Those data confirm the results of two other independent experiments which show that the optimum dose of endotoxin (LPSw O 128:B12 from *E. coli*) was 80–100 μg/kg and that between 10 and 25 μg/kg the endotoxin efficacy was not reproducible.

Oral treatment

OM-89 was administered per os to rats bearing microscopic or macroscopic carcinomatosis (600

Table 2. *In vivo* effect of OM-89 on the growth of macroscopic peritoneal carcinomatoses induced by colon cancer cells in rats, data of three independent experiments

Treatment (OM-89, 50 mg/kg)	Number of rats with carcinomatosis of the following classes					Effect of the treatment[a]	Volume of the ascites (ml/rat)		Effect of the treatment[b]
	0	1	2	3	4		Limit	Mean ± *SD*	
Without	0	1	0	5	4		1–52	21 ± 19	
With	4	1	3	2	0	$p < 0.01$	0–1	0 ± 0	$p < 0.001$
Without	0	1	1	2	6		75–83	79 ± 5	
With	5	1	3	0	1	$p < 0.01$	0–25	3 ± 8	$p < 0.001$
Without	0	2	5	3	0		1–12	3 ± 4	
With	5	4	1	0	0	$p < 0.01$	0–0	0 ± 0	$p < 0.001$

[a] Kruskal–Wallis test.
[b] Variance analysis.

Table 3. Dose effect of endotoxin on the growth of macroscopic peritoneal carcinomatoses induced by colon cancer cells in rats, data of a representative experiment

Treatment	Number of rats with carcinomatosis of the following classes					Effect of the treatment[a]	Volume of the ascites (ml/rat)		Effect of the treatment[b]
	0	1	2	3	4		Limit	Mean ± *SD*	
None	0	1	1	4	4		6–62	37 ± 22	
Endotoxin 100 μg/kg	2	2	4	2	0	$p < 0.01$	0–3	0 ± 0	$p < 0.001$
Endotoxin 10 μg/kg	0	4	3	3	0	NS	0–35	8 ± 11	$p < 0.05$
Endotoxin 1 μg/kg	0	1	4	3	2	NS	5–77	36 ± 25	NS

(NS) not significant.
[a] Kruskal–Wallis test.
[b] Variance analysis.

mg lyophilizate/kg, ie 60 mg of active substance/kg). In both experiments OM-89 was injected ip (50 mg of active substance/kg twice a week for 2 weeks) to other rats. Oral administration of OM-89 had no effect while ip administration prevented the growth of microscopic tumors in eight out of ten rats and induced the complete regression of macroscopic tumors in five out of ten rats.

Side-effects of OM-89

Intraperitoneal treatment. With OM-89 at a dose of 50 mg of active substance/kg, a transitory weight loss was observed in one experiment out of four. This was variable between animals, could be as great as 17% of the starting weight and disappeared by the end of treatment. In the other three experiments, the dose of 50 mg of active substance of OM-89/kg was well tolerated and did not cause weight loss.

Oral treatment. No side-effect was observed in two experiments with 60 mg of active substance of OM-89/kg administered daily for 21 days.

Discussion

The bacterial extract OM-89 was found to have antitumor properties. OM-89 when injected ip was able to induce the regression of macroscopic tumors in a model of colon cancer in rats. Those effects were reproducible and impressive: 46% of complete regression 6 weeks after the tumor cell injection whereas untreated controls had tumors of several centimeters. In this model, lentinan, a polyglucan extracted from mushroom *Lentinus edodes*, and endotoxin from *E. coli* were effective.[4,5] On the contrary, OK 432, a lyophilized extract from *Streptococcus pyogenes*, and other plant polyglucans were not effective (unpublished results). Lentinan and endotoxin were less effective and gave less reproducible results than OM-89. The traces of endotoxin in OM-89—0.0005% of the active substance, ie 0.25 μg/kg—could not be responsible for the antitumor effect of OM-89 since endotoxin from *E. coli* is no more effective at concentrations below 10–25 μg/kg.

In other experimental models, treatments of liver or lung metastases of colon carcinoma were reported: cyclic treatments with rat γ-interferon starting on day 7 significantly delayed the development of lung metastases induced by colon tumor cells, but had no influence on the growth of liver metastases.[6] In the same experimental model 2-amino-5-bromo-6-phenyl-4-pyrimidione (ABPP), a γ-interferon inducer, administered ip on days 0 and 1, reduced the number of liver metastases when counted on day 30. However, when ABPP was given on days 6 and 7, significantly more metastases were counted in the liver.[7] The combination of cyclophosphamide, tumor-infiltrating lymphocytes and IL-2, administered 8 days after the colon tumor cell injection, resulted in the long-term survival of all mice bearing liver metastases. The same treatment administered on days 12 and 14 after the colon tumor cell injection to mice bearing lung metastases significantly improved the life span.[8] It was recently reported that high doses of human IL-6 injected 3 days after the colon tumor cells during five or six consecutive days (three injections per day) significantly reduced the number of lung metastases counted at day 12.[9]

We have previously shown that the antitumor effects of lentinan and of endotoxin were due to different mechanisms. *In vivo*, the activity of lentinan is dependent on natural killer (NK) cells[10] while the endotoxins have an IL-1, TNF and T lymphocyte dependent activity.[11] Neither compound has an activity due to the direct cytotoxic effect of macrophages.[11] *In vitro*, OM-89 has a direct effect leading to the production of TNF, IL-1 and IL-6 by human monocytes and mouse-elicited peritoneal macrophages.[2,12] Those effects were due neither to the traces of endotoxins in OM-89 nor to synergy between OM-89 and endotoxins.[2] Although OM-89 does not have a direct effect on mouse lymphocyte proliferation it induces the IL-2 production of human peripheral blood mononuclear cells in the presence of phytohemagglutinin.[13] In addition, OM-89 augments human NK cell activity.[13] Thus the antitumor effect of OM-89 could be as follows: OM-89-activated macrophages produce TNF and IL-1, IL-1-activated T lymphocytes produce IL-2 which allows their proliferation, and then IL-1 and IL-2 induce the NK cell activity.

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