ANTICANCER ACTIVITY OF MYCOBACTERIAL CELL WALL-DNA COMPLEX (MCC) IN A MODEL OF RAT COLON CANCER PERITONEAL CARCINOMATOSIS

Mario C. FILION[1], Caroline MIGNARD[2], Olivier RAGUIN[2], Benoit FILION[1], Francis BICHAT[2] and Nigel C. PHILLIPS[1]

[1]Bioniche Life Sciences Inc., Montréal, Québec, Canada and [2]Oncodesign Biotechnology, Dijon, France

INTRODUCTION AND OBJECTIVES

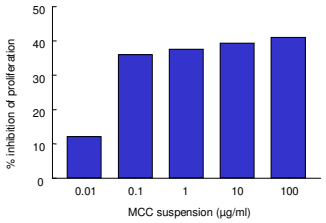
Mycobacterial cell wall-DNA complex (MCC) is a bifunctional anticancer agent that induces apoptosis in cancer cells and stimulates immune effector cells. The objective of this study was to evaluate the potential use of MCC as a localized treatment for peritoneal carcinomatosis. The antiproliferative activity of MCC towards rat DHD/K12/TRb colon cancer cells (PROb) *in vitro*, the *in vivo* recruitment of immune cells in the peritoneal cavity following repeated IP administrations of MCC and the *in vivo* antitumor activity of MCC against disseminated PROb colon cancer tumors in the peritoneal cavity of syngeneic BDIX rats were evaluated.

METHODS

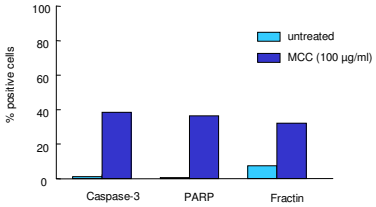
PROb cells were treated with MCC (0.01-100 µg/ml) for 72 h. Cell division was evaluated by MTT reduction; the induction and execution phases of apoptosis were evaluated by flow cytometry (antibodies recognizing the active form of caspase-3, and degraded PARP and Fractin respectively). Leucocyte numbers and leukocyte populations after MCC administration were determined in peritoneal washes after 9 IP administrations of MCC suspension (3 x weekly for 3 weeks). Disseminated peritoneal carcinomatosis was induced in female BDIX rats by the IP injection of 10^6 PROb cells. IP treatment with MCC (0.1 to 1000 µg/injection) was carried out 3 x weekly for 3 weeks starting on day 3 post-tumor cells injection (microscopic peritoneal nodes) or once on day 10 post-tumor cells injection (macroscopic peritoneal nodes). Survival and clinical signs and symptoms were determined daily for a period of 181d. Survival efficacy was determined as T/C% where T is the median survival time of treated rats and C is the median survival time of control rats. A T/C% value >125% was regarded as being significant (NCI criteria for anticancer activity). Animal experiments were performed according to ethical guidelines.

DIRECT ANTIPROLIFERATIVE AND APOPTOSIS-INDUCING ACTIVITIES OF MCC

Inhibition of PROb colon cancer cell division. MCC suspension inhibits the division of PROb colon cancer cells *in vitro*

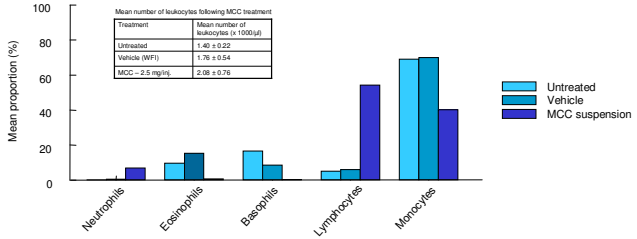


Induction of apoptosis in PROb colon cancer cells. MCC suspension induces apoptosis in PROb colon cancer cells *in vitro*



LEUKOCYTE POPULATIONS FOLLOWING MCC ADMINISTRATION

Leukocyte numbers and populations following treatment with MCC suspension. Mean proportion of neutrophils, eosinophils, basophils, lymphocytes and monocytes following 9 IP administrations of MCC



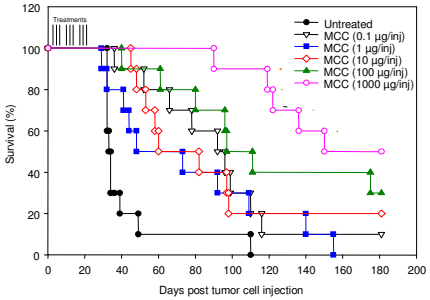
Mean number of leukocytes following MCC treatment

Treatment	Mean number of leukocytes (x 1000/µl)
Untreated	1.40 ± 0.22
Vehicle (WFI)	1.76 ± 0.54
MCC – 2.5 mg/inj.	2.08 ± 0.76

ANTITUMOR ACTIVITY OF MCC SUSPENSION IN BDIX RATS BEARING DISSEMINATED COLON CANCER PERITONEAL CARCINOMATOSIS

MICROSCOPIC PERITONEAL NODES (<1 mm)

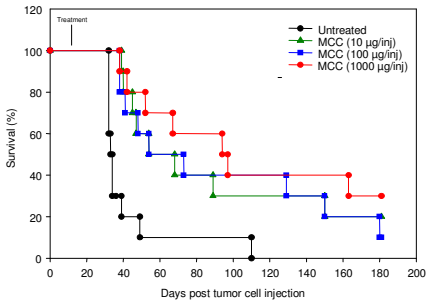
Repeat dose IP treatment with MCC suspension is effective against disseminated microscopic peritoneal nodes (treatment starting at day 3)



Treatment	Number of rats at D3	Survival rate at D181 (%)	Median survival times (days)	T/C (%)
Untreated	10	0	33.5	NA
MCC (0.1 µg/inj)	10	10	94	281
MCC (1 µg/inj)	10	0	60.5	181
MCC (10 µg/inj)	10	20	71	212
MCC (100 µg/inj)	10	30	104	310
MCC (1000 µg/inj)	10	50	165.5	494

MACROSCOPIC PERITONEAL NODES (1-5 mm)

Single dose IP treatment with MCC suspension is effective against disseminated macroscopic peritoneal nodes (treatment starting at day 10)



Treatment	Number of rats at D10	Survival rate at D181 (%)	Median survival times (days)	T/C (%)
Untreated	10	0	33.5	NA
MCC (10 µg/inj. D10)	10	20	61.5	182
MCC (100 µg/inj. D10)	10	10	63.5	189
MCC (1000 µg/inj. D10)	10	30	95.5	285

CONCLUSIONS

- MCC inhibits the proliferation and induces apoptosis in rat PROb colon cancer cells
- MCC is well tolerated following repeated IP administration
- MCC treatment increases the number of lymphocytes in the peritoneal cavity
- MCC significantly increases the survival time of colon cancer peritoneal carcinomatosis-bearing rats in a dose related manner
- MCC demonstrates a significant anticancer activity against both microscopic (day 3) and macroscopic disseminated peritoneal nodes (day 10) in rats
- MCC may have potential for the local treatment of disseminated peritoneal carcinomatosis

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