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Effect of malignant ascites on antioxidative potency of two tumoral cells-induced bone metastases: Walker 256/B and MatLyLu

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ABSTRACT

This study was undertaken to estimate antioxidative status of two malignant-mammary gland carcinoma (Walker 256/B) and malignant-prostate carcinoma cells (MatLyLu) disseminated in ascitic fluids. Malignant carcinoma cells (10^7 cells) were twice serially intraperitoneal injected in male Wistar rats to develop ascites. After 7 days, ascitic fluids were collected, and cells in suspension were usable for biological assays. Cellular lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) levels. Some antioxidant parameters: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were also assessed. Comparisons with control baseline (cells maintained in normal culture medium) were analyzed. TBARS levels were found to be significantly decreased in both ascitic cancer cells compared to the baseline except for in the ascite I of MatLyLu cells. On the other hand, SOD and CAT activities were found to be statistically increased in the two malignant ascitic passages. GSH-Px levels were elevated in the first and in the second ascitic passages ($p < 0.05$ and $p < 0.01$, respectively). Our results suggest that malignant ascites are associated not only with reduced levels of TBARS but also with increased antioxidant parameters, indicating the increasing antioxidant potency of two cancer cells during malignancies process.

1. Introduction

Bone metastases result in significant skeletal morbidity (hypercalcemia, pain, fractures, impaired mobility, spinal cord or nerve root compression and bone marrow infiltration). Median survival for patients with skeletal metastases is approximately 18–26 months after their initial diagnosis (Ries et al., 2005). Skeletal complications are a significant clinical concern in breast or prostate cancer patients with bone metastases, and preventing or delaying the occurrence of these events is an important objective of treatment. Breast cancer and prostate cancer cells presented a high metastatic potential and induced predominantly osteolytic lesions and osteosclerotic lesions, respectively.

Although there have been advances in radiotherapy, chemotherapy and surgery, the use of complementary therapies for many cancer diseases remains of key interest (Kasamon and Dawson, 2004). Several mechanisms of tumor cell treatments include the participation of lipid peroxidation (LPO) chains. Elsewhere, many antitumor drugs generate free radicals. However, free radicals processes could be involved in carcinogenesis. Some authors suggest a prominent disruption of endogenous and induced lipid peroxidation of most cancer cells (Birgit et al., 1992; El-akawi et al., 1996).

Oxidative stress has been suggested to play an important role in carcinogenesis. Antioxidant evaluation has recently sparked interest as tool for diagnosing diseases including cancers (Tezan et al., 2009). Moreover, the antioxidant status of cancer cells is known to be an important factor in tumor invasion and metastases, and there quantifications have become an important area of research (Badraoui et al., 2009).

Walker 256 and MatLyLu tumor cells have been used in animal models to study interactions between cancer and bone, they are very reproducible methods to induce osteolytic and osteosclerotic metastases, respectively (Blouin et al., 2005; Mao-Ying et al., 2006). Earlier reports, including our studies (Badraoui et al., 2009, 2008; Blouin et al., 2005; Mao-Ying et al., 2006), indicate that cancerous cells with bone trophicity could be obtained by their passaging intraperitoneally in rats to develop malignant ascites. In fact, two *in vivo* passages, of the above-cited cells, are needed to induce skeletal metastases in the receiver animal (Badraoui et al., submitted; Blouin et al., 2005). It is well known that ascitic fluids considerably modified cytokine content in presence of cancerous cells (Takagane et al., 1996), and to contain many physiologically active

Abbreviations: CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; LPO, lipid peroxidation; TBARS, thiobarbituric acid reactive substances; ROS, reactive oxygen species.

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elements such as growth factors that potentially promote tumor growth and metastasis complication (Koracevic et al., 2001). Furthermore, an up regulation of expression of adhesion molecules (ICAM-1, PECAM-1, and VCAM-1) in malignant ascites has been shown (Imitaz et al., 1998). However, to what extend malignant ascites, with high bone trophicity cells, could interfere with oxidizing parameters is little known and need to be further studied.

As little is known about the responses of antioxidant enzymes activities to cells with high osteophily and metastasizing ability, the aim of this work was to extend further relationships between malignant ascites and oxidative/antioxidative status in two tumor cells, Walker 256/B and MatLyLu, after two serially ascitic passages of 7 days each. Both cell lines are poorly immunogenic and are commonly used for experiments involving bone osteolytic and osteosclerotic metastasis respectively (Blouin et al., 2005, 2006). Comparisons with the baseline status, of cells maintained in normal culture medium, were studied for each cell line.

2. Materials and methods

2.1. Chemicals

Dexamethasone and 2-thiobarbituric acid (TBA) were purchased from Sigma–Aldrich (Illkirsh, France). 2,4-Dinitrophenylhydrazine (DNPH) was purchased from Fluka Co. (Buchs, Switzerland). Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute 1640 (RPMI 1640) medium, penicillin, streptomycin sulphate, and sodium pyruvate were obtained from Eurobio (Les Ulis, France). Nonessential amino acids and isoflurane were obtained from Cambrex (Walkersville, MD, USA) and AErrane (Baxter S.A., Belgium), respectively. Fetal calf serum was purshased from Seromed Biochrom (Berlin, Germany). Other chemicals were of analytical grade.

2.2. Cell line and culture conditions

Malignant Walker 256/B and MatLyLu (Metastatic Anaplasic Tumor metastazing to Lymph node and Lungs) cells were used. Both malignant cells were cultured at 37 °C in a humidified atmosphere using a water-jacketed 5% CO_2 incubator. Walker 256/B cells were grown in suspension in DMEM supplemented with 5% fetal calf serum, 100 UI/ml of penicillin, 100 μg/ml of streptomycin sulphate, 1% of nonessential amino acids, and 1 mM of sodium pyruvate. MatLyLu cells were grown in suspension in RPMI 1640 supplemented with 10% fetal calf serum, 100 UI/ml of penicillin, 100 μg/ml of streptomycin sulphate, and 250 nM of dexamethasone. For both malignant Walker 256/B and malignant MatLyLu carcinoma cell lines, 10^7 cells were passed intraperitoneally in Wistar rats to develop ascites. After 7 days, ascitic fluid was collected from each rat, and cells in suspension were usable for biological assays.

2.3. Cell viability

Viability of cells was determined by trypan blue exclusion assay. In fact, at the appropriate time, the cells were trypsined, neutralized, stained with 0.4% trypan blue solution, and then counted using a hemocytometer. Viability was about 99% before intraperitoneally injection of 10^7 malignant cells.

2.4. Animals and surgical procedure

Twenty-four male Wistar rats, of 8–10 weeks, were bred under well-controlled conditions (24 °C and a 12/12-h light/dark cycle). Malignant ascites in rats were obtained as previously described (Badraoui et al., 2009; Blouin et al., 2005). Seven days after cells injection, sufficient period to obtain cells with bone trophicity and metastatic ability (Badraoui et al., 2009; Blouin et al., 2005), animals were euthanized under anesthesia by an intraperitoneal injection of 8% chloral hydrate (400 mg/100 g BW). Fluid of ascites has been removed under sterile conditions, then, passed through a grid to remove tumor and/or stromal aggregates. After a gentle centrifugation (3000 × *g* at 4 °C for 10 min), the supernatant was discarded and cells were resuspended in an ammonium chloride solution in order to lyse red blood cells. The malignant cells were then washed several times by suspension in phosphate-buffered saline (PBS). The cell suspension has been used to assess oxidative–antioxidative status and reinjected for a second *in vivo* passage as above.

All procedures involving rat including their housing and care, the method by which they were killed, and the experimental protocols were conducted in accordance with the local Ethical Committee for the care and use of laboratory animals.

2.5. Biological assays

Biochemical assays were assessed, on the two malignant-mammary gland carcinoma (Walker 256/B) and malignant-prostate carcinoma cells (MatLyLu), as previously published (Badraoui et al., 2009, 2010).

The concentrations of thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation (LPO) were assessed by the method of Devasagayam and Tarachand (1987). Briefly, a volume of 0.2 ml of cancer cells was supplemented to a total of 2 ml the reaction mixture which consisted of 1 ml of 0.15 mol/l Tris–HCl buffer (pH 7.4) and 0.3 ml of 10 mM KH_2PO_4. Tubes were incubated at 37 °C with constant shaking. After 20 min, the reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid. The tubes were then shaken, 1.5 ml of TBA added and tubes were heated for 20 min in a boiling water bath. Then tubes were centrifuged at 3500 t/min for 2 min. TBA reacts with malondialdehyde (MDA) and MDA-like substances producing a pink pigment with an absorption maximum at 532 nm.

Catalase (CAT) activity was determined by the kinetic assay following the method of Beers and Sizer in which the disappearance of peroxide is monitored spectrophotometrically at 240 nm (Beers and Sizer, 1952). One unit of catalase is equivalent to the amount of protein necessary to decompose 1 μmol of H_2O_2 per minute.

Glutathione peroxidase (GSH-Px) was determined by a spectrophotometric method based on the protocol of Beutler and Matsumoto (1975). This method assay records the disappearance of NADPH at 340 nm. One unit of GSH-Px was equivalent to the amount of enzyme necessary to reduce 1 μM of NADPH per minute.

Superoxide dismutase (SOD) activity was determined by the method of McCord and Fridovich (1969). SOD activity was monitored spectrophotometrically at 505 nm. This method assay depends on SOD activity to inhibits cytochrome C reduction mediated by the $^{\bullet}O_2^-$ generated.

2.6. Protein estimation

Protein contents were estimated by the method of Bradford (1976) using bovine γ globulin as standard.

2.7. Statistical analysis

Results were expressed as the mean ± SEM. Differences between the groups were calculated by a one-way analysis of variance (ANOVA) and *post hoc* Tukey's multiple comparison test using the Prism software package (SPSS Inc., Chicago, IL). The value of $p < 0.05$ was considered significant.