Walker-256 Tumor: Experimental Model, Implantation Sites and Number of Cells for Ascitic and Solid Tumor Development

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Abstract: The Walker-256 tumor is an important experimental model that allow the development of therapies as the biological behavior of this tumor is similar that occur in humans. In front of the above considerations, the aim of this study was to describe the experimental model of Walker-256 tumor, identify the implantations sites as well as define a usual quantity of tumoral cells to induce the ascitic and solid tumor, according to the specialized literature. Were selected 45 articles using the keyword "Walker-256 tumor", free available. Were possible to observe that 58% (n=26) of the studies inoculate the tumor cells in the animals flank 33% (n = 15) in the tibia bone, 7% (n = 3) in the femur and 2% (n = 1) in the paw. The major quantitates of cells used were $8 \times 10^7$ (20%), $1 \times 10^5$ (13%), $1 \times 10^6$ (11%) and $2 \times 10^7$ (11%). After that, the site commonly used to inoculate was the flank and quantitate still a controversy, being $1\times10^5$ and $8\times10^7$ the concentrations more used.

Keywords: Walker-256 tumor; Cancer; Experimental model; Rats.
INTRODUCTION

Is expected that 14 million of people develop cancer each year, and this number must increase to more than 21 million until 2030. This disease is responsible for almost one in each six deaths worldwide. Each year, 8.8 million of people died from cancer especially in low income countries.

Among the causes of death by cancer is cachexia, responsible by 20% of death. This complication in oncologic patients cooperate to a worse prognostic, lower survival, alterations quality of life, deterioration in functional capability, as well as significantly contribute to toxicity induced by chemotherapy.

Is known that the conventional treatment for cancer is chemotherapy. However, it case diverse collateral effects and are not efficient in complete remission of tumor. Therefore, several studies are developed searching for new substances that can substitute the conventional method. Thus, it is necessary to use experimental models that corresponds more to the reality of individuals affected by this disease.

Walker-256 tumor is a model that allow this situation. This model is possible to observe the three carcinogenesis stages: initiation, promotion and progression in a brief period of 12-16 days. In addition, the Walker-256 tumor exhibit aggressive biological behavior, locally invasive, with high metastasis capacity.

This tumor is used in studies for breast cancer, bone and paw tumors, it has accelerated growth, causing cachexia and oxidative stress, and still has a high metabolic demand, similar to what occurs with cancer patients.

The present study had as objective describe the experimental tumor model Walker-256, identify the implantation sites, as well as define a quantity of usual tumoral cells to induce the ascitic and solid tumor, according to the literature.

MATERIAL AND METHODS

Were included experimental studies that used the tumor model Walker-256 in this review. The search were performed by the Pubmed database, using the keyword: Walker-256. Were considered the articles free available, published between 2012 and July/2018. The exclusion criteria were: (1) do not fit in criteria described above; (2) literature review; (3) case study; (4) retrospective and observational studies; (5) do not describe quantitate of cells used to induce solid tumor.

The search resulted in 1253 articles, to extract the data were evaluated the titles and abstracts of all articles. All abstracts that reported sufficient information according to the inclusion and exclusion criteria were selected. The eligibility step were excluded studies that do not describe the quantity of cells used to induce solid tumor. At the end of assessment, forty studies meet the inclusion and exclusion criteria and were evaluated (Figure 1). Were included a few studies that do not meet the criteria, but they are the basis for this theme.
RESULTS

At this research, were included 45 articles according to the selection criteria described at the material and methods section. In Table 1 are listed the selected articles to the review, with description of quantity of cells used and implantation sites of Walker-256 solid tumor.

Table 1. Site of implantation and inoculated number of cells for induction of solid tumor of Walker-256

<table>
<thead>
<tr>
<th>Tumor implantation site</th>
<th>Objectives</th>
<th>Inoculated number (cells / rat)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flank</td>
<td>To investigate the effects of aerobic exercise training starting at adolescence, on Walker 256 tumor growth and insulin secretion in adult rats</td>
<td>$8 \times 10^7$</td>
<td>MOREIRA et al., 2018$^{15}$</td>
</tr>
<tr>
<td></td>
<td>To evaluate whether obese adult rats that were chronically treated with an antidiabetic drug, glibenclamide, exhibit resistance to rodent breast carcinoma growth</td>
<td>$8 \times 10^7$</td>
<td>FRANCO et al., 2017$^{16}$</td>
</tr>
<tr>
<td></td>
<td>To identify mechanisms of inflammatory response in atrophy in cancer cachexia</td>
<td>$2 \times 10^7$</td>
<td>HENRIQUES et al., 2017$^{17}$</td>
</tr>
<tr>
<td></td>
<td>To investigate the antitumor activity of the soluble fraction of polysaccharides, extracted from cabernet</td>
<td>$2 \times 10^7$</td>
<td>STIPP et al., 2017$^{10}$</td>
</tr>
</tbody>
</table>
To investigate the pioglitazone effects, isolated or associated with insulin, on insulin resistance, cachexia and metabolic disorders in cancer animal model

To evaluate the in vivo antitumor effects and toxicity of a new Ru(II) compound, cis-(Ru[phen][2][ImH][2]2+ (also called RuphenImH [RuC]), against Walker-256 carcinosarcoma in rats

To analyse the modulatory effect of a leucine-rich diet on direct and indirect tumor-induced placental damage

To determine the effect of tumors on interstitial cells of Cajal in the rat jejunum and to investigate the effect of 2% L-glutamine on interstitial cells of Cajal and tumor-induced changes

To assess the effect of endostatin combined with a small dose of 32 P-colloidal in vivo

To assess antioxidant effects of açaí seed extract on anorexia-cachexia induced by Walker-256 tumor

To provide insight into adipocyte involvement in inflammation along the progression of cachexia

To evaluate the metformin on Walker-256 tumor evolution and also on protein metabolism in gastrocnemius muscle and body composition

To evaluate whether leucine supplementation ameliorates cachexia in the heart

To evaluate whether a leucine-rich diet affects metabolomic derangements in serum and tumor tissues in tumor-bearing Walker-256 rats

To evaluate the effect of dietary supplementation with 20 g/kg L-glutamine on the intrinsic innervation of the enteric nervous system in healthy and Walker 256 tumor-bearing Wistar rats during the development of
experimental cachexia

To investigate the pharmacokinetics profiles of ginsenoside Rg and ginsenoside Rh after oral administration of pure ginsenoside Rg were administered, and compare the difference of the pharmacokinetics profiles between normal and Walker 256 tumor bearing rats

To investigate the effect of fish oil supplementation on apoptosis protein expression in Walker 256 tumor bearing rats

To evaluate the in vivo antitumor actions and toxicity of the dichloromethane fraction of *Moquiniastrum polymorphum* subsp. *floccosum* (formerly *Gochnatia polymorpha* ssp. *floccosa*), composed of sesquiterpene lactones, against Walker-256 carcinosarcoma in rats

To investigate the effects of celecoxib and ibuprofen, both non-steroidal anti-inflammatory drugs, on the decreased gluconeogenesis observed in liver of Walker-256 tumor-bearing rats

To evaluate the in vivo antitumor actions and toxicity of the dichloromethane fraction of *Moquiniastrum polymorphum* subsp. *floccosum* (formerly *Gochnatia polymorpha* ssp. *floccosa*), composed of sesquiterpene lactones, against Walker-256 carcinosarcoma in rats

To investigate the effects of celecoxib and ibuprofen, both non-steroidal anti-inflammatory drugs, on the decreased gluconeogenesis observed in liver of Walker-256 tumor-bearing rats

To test the effect of metformin on the tumor growth in rats with metabolic syndrome

To investigate the effect of fish oil supplementation on tumor growth, cyclooxygenase 2, peroxisome proliferator-activated receptor gamma, and RelA gene and protein expression in Walker 256 tumor-bearing rats

To evaluate gluconeogenesis from alanine, pyruvate and glycerol, and related metabolic parameters in perfused liver from Walker-256 tumor-bearing rats on days 5, 8 and 12 of tumor development

To investigate the effect of infliximab, an anti-tumor necrosis factor a monoclonal antibody, on the...
progression of cachexia and several metabolic parameters affected by the Walker-256 tumor in rats

<table>
<thead>
<tr>
<th>Study</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>To describe effects of the resistance exercise training upon adipose tissue inflammation in cachexia</td>
<td>3 x 10^7</td>
<td>DONATTO et al., 2013^{33}</td>
</tr>
<tr>
<td>To describe set point of weight loss and how the different visceral adipose tissue depots contribute to this symptom</td>
<td>2 x 10^7</td>
<td>BATISTA JR et al., 2012^{34}</td>
</tr>
<tr>
<td>To investigate the effects of electroacupuncture on mechanical allodynia and cellular immunity of cancer-induced bone pain rats, and to further explore the potential mechanism</td>
<td>3 x 10^5</td>
<td>LIANG et al., 2018^{35}</td>
</tr>
<tr>
<td>To investigate the role of NF-κB in CIBP by regulating MCP-1/chemokine CC motif receptor-2 (CCR2) signaling pathway.</td>
<td>1 x 10^6</td>
<td>WANG et al., 2018^{36}</td>
</tr>
<tr>
<td>To investigate whether P2Y12R is involved in the establishment of cancer-induced bone pain model by inoculating Walker 256 breast cancer cells in the tibia and to examine the effect of P2Y12R antagonist on spinal neuroimmune activity in a cancer-induced bone pain model</td>
<td>2 x 10^7</td>
<td>LIU et al., 2017^{37}</td>
</tr>
<tr>
<td>To investigate the role of Suppressor of cytokine signaling 3 in dorsal root ganglion in the development of cancer-induced pain</td>
<td>4 x 10^5</td>
<td>WEI et al., 2017^{38}</td>
</tr>
<tr>
<td>To assess the antinociceptive effect of Tanshinone IIA on cancer-induced bone pain</td>
<td>5 x 10^2</td>
<td>HAO et al., 2016^{39}</td>
</tr>
<tr>
<td>To investigate whether spinal CCR5 and its downstream PKCy pathway is involved in the maintenance of cancer-induced bone pain</td>
<td>1 x 10^5</td>
<td>HANG et al., 2016^{40}</td>
</tr>
<tr>
<td>To investigate the mechanisms underlying the anti-nociceptive effect of minocycline on bone cancer pain in rats</td>
<td>4 x 10^5</td>
<td>SONG et al., 2016^{4}</td>
</tr>
<tr>
<td>To investigate whether the lysophosphatidic acid receptor 1 and Rho / ROCK signaling are involved in cancer-induced bone pain</td>
<td>2 x 10^5</td>
<td>PAN et al., 2016^{41}</td>
</tr>
<tr>
<td>Create a viable prolonged treatment for bone cancer pain</td>
<td>5 x 10^5</td>
<td>XU et al., 2015^{42}</td>
</tr>
</tbody>
</table>
To determine the efficacy of a calpain inhibitor on bone resorption and behavioral responses to pain *in vivo* in intratibial tumor injected cancer-induced bone pain rats

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calpain inhibitor</td>
<td>$1 \times 10^5$</td>
<td>ZHU et al., 2015$^{43}$</td>
<td></td>
</tr>
</tbody>
</table>

Examine the potential of the spinal sigma-1 receptor in the development of cancer-induced bone pain

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigma-1 receptor</td>
<td>$2 \times 10^5$</td>
<td>WU et al., 2016$^{44}$</td>
<td></td>
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</tbody>
</table>

Examine the potential role of the spinal PKA/CREB signaling pathway in the development of bone cancer pain

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKA/CREB signaling</td>
<td>$1 \times 10^5$</td>
<td>HANG et al., 2013$^{45}$</td>
<td></td>
</tr>
</tbody>
</table>

To investigate whether the chemokine receptor 5 and C-kinase receptor pathway is involved in the maintenance of cancer-induced bone pain

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemokine receptor</td>
<td>$1 \times 10^5$</td>
<td>HANG et al., 2013$^{46}$</td>
<td></td>
</tr>
</tbody>
</table>

To investigate the effects of intrathecal injection with lipoxin and related analogues on cancer-induced bone pain in rats

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrathecal injection</td>
<td>$1 \times 10^8$</td>
<td>HU et al., 2012$^{47}$</td>
<td></td>
</tr>
</tbody>
</table>

To investigate the role of c-jun N-terminal kinase pathway in the spinal cord in cancer-induced bone pain

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-jun N-terminal kinase</td>
<td>$3,5 \times 10^5$</td>
<td>WANG et al., 2012$^{48}$</td>
<td></td>
</tr>
</tbody>
</table>

Femur

To investigate the hypothesis that urinary levels of N telopeptide (NTx) can be used to predict the anti-nociceptive responses of zoledronic acid and paclitaxel on bone metastases in a rat model

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary levels</td>
<td>$1 \times 10^5$</td>
<td>GUI et al., 2015$^{49}$</td>
<td></td>
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</table>

Compare the effects of ibandronate and paclitaxel on bone structure and its mechanical properties and biochemical turnover in resorption markers using an immunocompetent Walker 256-Sprague-Dawley model, which was subjected to tumor-induced osteolysis.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone structure</td>
<td>$2,5 \times 10^6$</td>
<td>CHUNG et al., 2015$^5$</td>
<td></td>
</tr>
</tbody>
</table>

Establish a model of femoral bone cancer

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral bone cancer</td>
<td>$1 \times 10^5$</td>
<td>GUI et al., 2013$^{50}$</td>
<td></td>
</tr>
</tbody>
</table>

Paw

To investigate the effects of crotoxin on Walker 256 tumor growth, the pain symptoms associated (hyperalgesia and allodynia), and participation of endogenous lipoxin A4

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Authors</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crotoxin effects</td>
<td>$1 \times 10^6$</td>
<td>BRIGATTE et al., 2016$^6$</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

History of Walker-256 tumor

George Walker observed firstly in 1928 the Walker-256 tumor spontaneously in the region of mammary gland of a pregnant albino rat, which regressed completely during the lactation period. But it grew again, after the weaning of the offspring. Thus, this was the first researcher to perform the implant using these tumor cells, through fragmentation 51.

Subsequently, the technique was improved and the tumor cell line is easily implantable, specific for mice and grows rapidly in the host animal. The cells are maintained in the laboratory by means of weekly passages into intraperitoneal cavity of rats, when necessary the solid tumor is induced by subcutaneous or muscle and become palpable about four days post-implant and can grow to a mean diameter of 20-30 mm within 8 days 4,13,23,27,52.

Cell maintenance

Walker-256 tumor cells are maintained by weekly passages of the intraperitoneal cavity of rats of both sexes. After the intraperitoneal application the survival of animal is of seven days 27. For this procedure it is necessary that the animal be anesthetized and subsequently submitted to euthanasia according to the ethical principles affirmed by the Brazilian College of Animal Experimentation 53 and by the Declaration of the Rights of the Animals 54.

After euthanasia, the cells are harvested from the abdominal cavity, centrifuged, resuspended in phosphate-saline buffered, saline solution or Hank's balanced saline solution and performed the cell viability test by the Trypan blue exclusion assay in Neubauer’s chamber. Subsequently, the cells are inoculated into a second animal intraperitoneally, until the application the cells need to be refrigerated 6,10,27,31,44,45.

The ascites tumor is neither visible nor palpable. The ascitic fluid is hemorrhagic, so some authors such as Martins et al. 13 and Stipp et al. 10 reported using the solution of ethylenediaminetetraacetic acid (EDTA) as anticoagulant in the collection because of the blood present.

Few studies describe the quantity of cells used for ascites tumor induction, among the 45 papers analyzed, only 10 cited the amount used. The most commonly used amounts were 1 x 10^7 (70%) and 2 x 10^7 (30%).

Solid tumor implantation sites

The implant is performed after the Trypan blue exclusion test in the Neubauer chamber. The cells are resuspended in phosphate-saline buffer, saline solution or Hank's balanced saline and applied at the sites determined by the studies 6,10,13,27,45 added the antibiotic cell suspension (benzylpenicillin and benzetacil) in order to avoid microbial contamination.

It was observed in Table 1 that 58% (n = 26) of the studies inoculated the cells in the flanks of the animals, 33% (n = 15) in the tibia bone, 7% (n = 3) in the femur and 2% (n = 1) in the paw, using the subcutaneous via.

Tumors inoculated on the tibia and femur seek to elucidate the mechanisms and treatments related to cancer-induced bone pain. It was observed that the largest number of studies used the flank because it did not specify the primary site related to the human, it is worth mentioning that the implant is performed on both the right and left flanks (Figure 2).
Figure 2. Implantation of Walker-256 tumor cells by subcutaneous injection into flank pathway for induction of solid tumor

Cell inoculation for induction of solid tumor

According to Table 1 it was observed that the main quantity of cells used were $8 \times 10^7$ (20%), $1 \times 10^5$ (13%), $1 \times 10^6$ (11%) and $2 \times 10^7$ (11%). Thus, we observed that there is no consensus among the articles regarding the quantity of cells to be applied for the induction of solid tumors.

According to this review it was possible to verify that the experimental period was of 12 to 16 days, not obtaining a standard in the amount of days and dose for each site of implantation.

CONCLUSION

This review allowed to know the Walker-256 tumor and its peculiarities. Thus, we understood that for the ascites tumor induction the quantity $1 \times 10^7$ and $2 \times 10^7$ are used, according to the literature. It is also inferred that the main site of implantation of this cell line for induction of solid tumor is the flank and the amount of cells is not yet defined. However, the most used quantities are $8 \times 10^7$ and $1 \times 10^5$. Suggesting that this number may vary according to the aggressiveness of the cells and experimental design.

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REFERENCES


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