

# İnokulasyonun multifokal tekniği kullanılarak walker 256 tümörüne histopatolojik olarak yaklaşımlar

## Histopathological aspects of walker 256 tumor using the multifocal technique of inoculation

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### ÖZET

Kanser, dünyada büyük ekonomik ve sosyal kayıplara neden olan ciddi felaketlerden birisi olarak tanımlanmaktadır. Sağlığı bozan bu vakaların artışı göz önüne alındığında, mevcut değişen etkinlik ile sık sık yan etki gösteren kemoterapi protokolleri, dünyada kanser terapisi ve kanserden koruyucu yeni stratejilerin geliştirilmesini gerekliliği ortaya çıkmaktadır. Walker 256 tümörünün kanser çalışmalarında kullanılan en yaygın deneysel tümör olmasına rağmen bu konuda sağlık personelinin çok az bilgiye sahip olduğu iyi bilinen bir gerçektir. Bu çalışmanın amacı; deneysel tümörleri gözden geçirmek ve aynı zamanda sağlık çalışanlarının bilgi düzeylerini derinleştirerek artırmaktır.

**Anahtar Kelimeler:** Kanser, walker tümörü, deney

### ABSTRACT

Cancer has been considered one of the most serious calamities all over the world, producing tremendous economic and social losses. Considering the increasing incidence of these health disturbances, the variable efficacy and frequent adverse events commonly notified with the existing chemotherapy protocols and the new events currently in progress in the world it's urgent to develop new strategies to prevent and treat cancer. It is well known that walker 256 tumor is the most common experimental tumor model to study cancer but public health personnel still have little information about it. The aim of this study is not only to review the important aspects of this experimental tumor but also to increase the knowledge and comprehension about it among health professionals.

**Key Words:** Cancer, walker tumor, experiment

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## INTRODUCTION

Cancer is defined as a cell disturbance characterized by alterations in DNA duplication, resulting in disordered cell growth and proliferation. Numerous chemical and biological agents such as hormones, cytokines, antineoplastic and tumoricidal agents which are connected to monoclonal antibodies are used in the treatment of cancer (1, 2).

Substances used in the treatment of cancer inhibit cell growth by different mechanisms of action. They exert their cytotoxic action not only on cancer cells but also on normal cells which have rapid replicative capacity such as the cells of bone marrow, skin, gastrointestinal epithelium and embryonic structures. For this reason most patients present with nausea, vomiting, and skin reactions such as hyperpigmentation and alopecia (1, 2).

Throughout human history, cancer has been considered as one of the most serious calamities. All over the world it is considered one of the main causes of death and a problem of public health. In 1985 mortality caused by cancer was 6% and in 1997 it reached 9% in developing countries and 21% in under developed countries (3).

Researchers have great interest in the development of new strategies to prevent and treat cancer. For ethical reasons, studies on cancer have been developed with animal models and the walker 256 tumor is the most common experimental tumor model (4). Although the results of experimental studies, especially with rodents, have to be carefully analysed before being transferred to clinical practice, they allow the observation of important effects like: anemia, clinical conditions, tumoral growth, survival and the results of new therapeutic strategies.

In this paper it is aimed to review the histopathological aspects, evolution, metastasis and metabolism of walker 256 tumor, using the multifocal technique of inoculation.

## Histopathology of Walker 256 Tumor

Walker 256 tumor is a mammary gland neoplasm of female rats, firstly identified by George walker in 1928 (4, 5). In this experimental investigation, Walker tumor cells have been inoculated in sites that are different from the original site of their inoculation such as: intradermal, subcutaneous, intracardiac, intrapleural, intraperitoneal, splenic, intravascular and intratracheal. Walker tumor cells are easy to manipulate, which facilitates their inoculation in different types of tissues (4, 5).

In the identification process, the continuous observation of pregnant rats allowed the visualization of palpable mass in the left lower abdominal quadrant. During lactation an almost completely regressed tumor was observed to follow a rapid growth, reaching a greater size than its initial dimensions (5).

Although the walker 256 tumor has been recognized by its aggressiveness, rapid growth, lymphatic and hematogenous dissemination along with frequent lung metastasis, its origin and histopathologic nature is not clear since authors suggest different features e.g. carcinomatous, adenomatous, lymphoid and even hematogenous (5-8).

Earle (5), after obtaining several types of cells from Dr. Walker's laboratory, carried out a histological study where he describes the characteristics of growth and microscopy of this tumor. He confirmed the adenomatous character of it at the initial phase and the adenocarcinomatous character at the final stage, describing subsequent histopathological stages of the evolution. Tumor sections with hemorrhagic necrotic central aspects were stated as evidences of carcinomatous transformation in this study. Sections of tumors evidenced a hemorrhagic necrotic central aspect, with carcinomatous appearance (5).

In a histochemical enzymatic study, the walker 256 tumor showed an intense focal acid phosphatase activity suggesting that cells were of hematopoietic origin.

So, the concept that these cells reflect epithelial origin is not substantiated by the phenotype. When implanted, this tumor grows with the morphology of a carcinosarcoma, exhibiting 2 to 3 cell types, forming independent cell patterns. In microscopic exam, the patterns of carcinoma or sarcoma appear as individual entities (8).

This study suggested a hematopoietic origin for these cells since they had monocytoid characteristics. This concept is sustained by the growth properties of these cells which occur in non-adherent groupings of reminiscent cells from a culture of leukemia lymphoid cells, in a more evident way than layers of epithelial cells (7, 8).

The walker 256 tumor in rats (WS cells) is sensitive to cisplatin and chlorambucil in contrast to the lineage WR that is derived from the exposure of WS lineage to chlorambucil. The WS cells seem to present a normal sensitivity to these agents. Studies demonstrate that the number of chromosomes of WS and WR lineages present little differences in triploidy and tetraploidy. WS and WR lineages of walker tumor cells have hematopoietic origin and absence of epithelial cell markers (5-8).

#### **Metastasis of Walker 256 Tumor**

The information about the frequency and distribution of walker 256 tumor metastasis are controversial (6, 8). Buck in 1937, described the metastatic dissemination in abdominal cavity associated with visceral invasion, after the introduction of walker 256 tumor cells in peritoneal cavity of rats. It is believed that the dissemination is made by lymphatic circulation. In this case the regional and abdominal lymph nodes were mostly, and the thoracic and axillary lymph nodes were rarely, affected. Affected lymph nodes present higher volume, but their limits are maintained (8).

In multifocal and subcutaneous inoculations both regional and distal lymph node metastases and distant metastases to lungs, liver and kidneys were

observed. A few thoracic and abdominal metastatic lymph nodes have been described in the uni and bifocal subcutaneous inoculation (4, 9-12).

The lung metastasis can occur after the inoculation of endotracheal tumoral cells and the dissemination follows the lymphatic standard. The involvement of lung lymphatic vessels can result in clinical sub-acute signs and symptoms. The pathogenesis of lung hypertension and cardiac insufficiency in carcinomatous lymphangitis is still controversial, and various theories have tried to explain its involvement. Among them are the possibility of extrinsic compression of blood vessels by lymphatic vessels and invasion of arterial wall by neoplastic cells (9, 10, 13).

Liver metastasis and cutaneous growth of walker 256 tumor have a similar appearance to various malignant tumors. Cytoplasmic pseudopod formations were observed between the tumor and hepatic cells, when a metastasis is present. On the other hand, the contact of hepatic and tumoral cells could be some sort of confrontation of both cells (13). The nutritional supplementation with 6% arginine possibly inhibits the metastatic dissemination of tumoral cells in experimental models of ascitic and solid forms of Walker 256 tumor. The nutritional supplementation with L-arginine in animals with solid walker 256 tumor showed a beneficial action of this substance in the prevention of metastasis of this same tumor (14-16). The analyses of ascitic walker tumor also showed a positive effect with arginine supplementation. Reduction was observed of the aggressive character of the tumor and the intensity of metastatic dissemination (16).

Scientific literature has shown that surgery has controversial effects on cancer patients, since it reduces the immunological defenses of the host pre-disposing metastatic dissemination and tumor development. Fluoxetine (anti-depressant) has attested to be able to prevent immunodepression after surgery (17). Studies were conducted to prove

the effect of fluoxetine in metastatic dissemination of walker tumor in rats submitted to laparotomy. It was observed that fluoxetine is able to reduce a number of metastatic lymph nodes in lungs of post operated rats and response was even more significant when administered two hours after surgery. Good results were also obtained during the survival period of the animals. The administration of fluoxetine before surgical stress resulted in a decrease of lethality rate. These results are attributed to the protective action of fluoxetine in the immunological system, even though various publications describe Fluoxetine as having cancerous action (17).

#### **The Evolution Of Walker 256 Tumor in Wistar Rats, Using The Multifocal Technique of Inoculation.**

The walker 256 tumor has been extensively used in oncologic physiopathology. This tumor develops without any functional disturbance for a period of time that cannot be predicted (in an individual basis), being interrupted by subtle and rapid homeostatic alterations evidenced by anorexia, immunological and electrolytic alterations to finish up in obit.

The systemic effect observed presents great individual variability. Investigations were carried out using techniques that reduce these variations during the inoculation of walker 256 tumor in unilateral site, two sites (bilateral) and four sites of inoculation (multilateral) (9, 11, 17).

The multifocal inoculation presented more advantages in relationship to uni and bilateral techniques by the fact that this method produces greater synchronism in the different phases of tumor growth, minimizing the effects of variations caused by individual response obtained by other methods.

The differences found in relation to other methods of inoculation refer mainly to the phases of tumoral development and the beginning of metastasis. This is probably due to the high level of mediators that are able to surpass the capability of protective mechanisms.

The multifocal technique followed a typical standard evolution: the initial period free of systemic detectable effects (sub clinical period, SCP), then a subtle interruption by a symptomatic (clinical period, CP) where it is possible to determine a moderate clinical period (mCP) and finally a severe clinical period (gCP). Four points were established for synchronizing the data, to determine the different evolution stages of the disease (9, 11, 17).

- Day of inoculation.
- Last day free of systemic effects, defined by the beginning of anorexia.
- Last day of moderate systemic effects marked by the worsening and acuteness of anorexia followed by loss of weight and water balance.
- Last day of survival.

These four points established the limits and defined phases such as CSP, mCP and gCP.

During the clinical period, the mCP, a slow progression of anorexia was observed, accompanied by weight gain due to fluids retention. The results showed a significant and early decrease of urinary excretion during mCP when compared to SCP. The renal sites involved were studied in animals by measuring the clearance of sodium, creatinine and lithium, indicating an initial rise in the absorption of sodium even in the proximal and post-proximal tubule which was partially compensated by the increase of rate in the glomerular filtration and by the reduction of fractionated proximal reabsorption although it is observed a significant retention of sodium and fluids. The duration was  $4.2 \pm 0.2$  days.

The terminal phase (gCP) was characterized by a progressive anorexia and weight loss. Other presented signs and symptoms were alopecia, increased urine osmolarity, hypodynamia, pallor (without evident loss of blood), priapism, scrotal retraction, urinary incontinency and retention of sodium. The medium duration of this phase was  $8.9 \pm 0.5$  days. This phase culminated with a decline in creatine clearance, suggesting a significant reduction of renal function.

Using the multifocal subcutaneous technique of inoculation, it was shown that disturbance in the homeostatic central mechanisms, caused by humoral factors can be the cause of cancer cachexia. The multifocal inoculation method produces high central levels caused by humoral factors and produces high levels of mediators that rapidly surpass the capability of protective mechanisms. The nature of humoral mediator needs additional investigations (17).

#### **Metabolism of Walker 256 Tumor**

There are important alterations in protein metabolism of rats with walker 256 tumor. These alterations are the result of protein growth in great tumors and the significant reduction of protein synthesis in the muscles and the whole body (18). The nutritional supplementation with 4% and 6% arginine were able to stimulate the metabolism of amino acids in rats with ascitic tumors. This was evident by the significant rises of plasmatic levels of arginine, ornitine, citruline, proline and histidine, when compared to control group (rats with solid or ascitic walker 256 tumor, that did not receive dietetic supplementation) (15). A decrease in plasmatic levels of amino acids in animals with walker 256 tumor, without dietetic supplementation is probably due to the utilization of amino acids by the malignant tumor, since the tumor needs of amino acids as a nitrogen source for protein synthesis and as a source of energy for the metabolism through gluconeogenesis (14, 15).

The accelerated growth of the tumor does not occur as a consequence of the rise of protein synthesis in cells, but, by the reduction of degradation mechanisms of tumoral proteins. This behavior favors therapies which act in the catabolism of tumoral proteins such as immunotherapy or the manipulation of amino acids and nutrients, associated with conventional therapies (14, 15).

The ingestion of liquids by rats with developing walker 256 tumor increased about 65%. Only 30% of the consumption of liquid is attributed to the

necessity related to water retention in the whole body, mainly in tumor, and by the increase of urinary osmolarity. 35% of the remaining water represents an increase in the turnover of free water and it appears as an increase in urine volume. The hypertrophied adrenal cortex resulting in an excessive secretion of aldosterone and retention of sodium stands for a hormonal factor that justifies this increase in liquid ingestion (16). In the first phase of tumor growth, a super-hydration of host tissues was observed, followed by a discrete terminal dehydration and progressive reduction of sodium content in the urine. Rettori et al., (11) observed that the retention of sodium occurs when tumor represents 10% or more of body weight and the excretion are normalized with the extinction of the tumor. The excretion of potassium remains unchanged during tumoral development.

The increase of amino acid consumption by tumoral cells stimulates the intestinal absorption of leucine and metiotine, essential amino acids to walker 256 tumor growth (1). Experiments with rats CO/COBS, with walker 256 tumor, showed a significant increase in the absorption of both amino acids, when compared to control group. This increase was even more significant in recently weaned rats. This probably occurs as the answer to accelerated tumor growth in another group of animals other than rats, when compared to the group of adult rats. Other possible explanations are the decreases in number and/or size of microvilli and a reduction in number of the enterocytes and/or its membrane carriers (1, 13).

Unlike the faith of amino acids, a decrease is observed in the absorption of glucose in animals with walker 256 tumor. This phenomenon is more revealing in adult rats and can affect the tumoral growth that probably obtains energy by processes similar to hepatic gliconeogenesis. In parallel to tumoral development, Gomes-Marcondes et al; (1) also observed a more pronounced decrease in nitrogen balance in adult rats (1, 18).

The walker 256 tumor also interferes with bone metabolism (19). The variant S of this carcinosarcoma, is able to develop bone metastasis and was responsible for bone alterations in young rats, a reduction in seric levels of  $\beta$ -estradiol and increase of reticuloendothelial activity of the immunological system. These results can be used to investigate the therapeutic strategies against osteoporosis, menopause or hypoovarianism, since bone alterations in rats with walker 256/S tumor, are similar to the alterations observed in this pathology. Loss of bone mass, increase of osteoclasts and reduction of the osteoblasts number in the femur of rats with walker 256 carcinosarcoma, are also observed. The molecular mechanisms remain unknown.

## CONCLUSION

Different inoculation techniques are used with walker 256 tumor as described in the scientific publications. That information has been facilitating the use of walker 256 tumor to investigate clinical conditions of cancer, chemotherapeutic agents, nutritional supplements and many other kinds of treatments. As walker 256 tumor cells are easy to cultivate, easy to inoculate and rarely present spontaneous remission, it is a good experimental model for the study of cancer.

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